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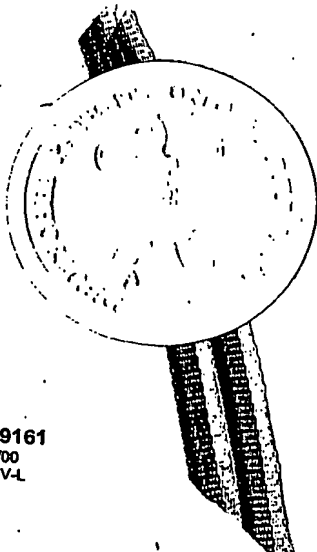
Bezeichnung: Method and nucleic acids for the improved treatment of breast cell proliferative disorders.

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München, den 2. November 2005
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Method and nucleic acids for the improved treatment of breast cell proliferative disorders

Field of the Invention

The levels of observation that have been studied by the methodological developments of recent years in molecular biology, are the genes themselves, the translation of these genes into RNA, and the resulting proteins. The question of which gene is switched on at which point in the course of the development of an individual, and how the activation and inhibition of specific genes in specific cells and tissues are controlled is correlatable to the degree and character of the methylation of the genes or of the genome. In this respect, pathogenic conditions may manifest themselves in a changed methylation pattern of individual genes or of the genome.

DNA methylation plays a role, for example, in the regulation of the transcription, in genetic imprinting, and in tumorigenesis. Therefore, the identification of 5-methylcytosine as a component of genetic information is of considerable interest. However, 5-methylcytosine positions cannot be identified by sequencing since 5-methylcytosine has the same base pairing behaviour as cytosine. Moreover, the epigenetic information carried by 5-methylcytosine is completely lost during PCR amplification.

A relatively new and currently the most frequently used method for analysing DNA for 5-methylcytosine is based upon the specific reaction of bisulfite with cytosine which, upon subsequent alkaline hydrolysis, is converted to uracil which corresponds to thymidine in its base pairing behaviour. However, 5-methylcytosine remains unmodified under these conditions. Consequently, the original DNA is converted in such a manner that methylcytosine, which originally could not be distinguished from cytosine by its hybridisation behaviour, can now be detected as the only remaining cytosine using "normal" molecular biological techniques, for example, by amplification and hybridisation or sequencing. All of these techniques are based on base pairing which can now be fully exploited. In terms of sensitivity, the prior art is defined by a method which encloses the DNA to be analysed in an agarose matrix, thus pre-

venting the diffusion and renaturation of the DNA (bisulfite only reacts with single-stranded DNA), and which replaces all precipitation and purification steps with fast dialysis (Olek A, Oswald J, Walter J. A modified and improved method for bisulphite based cytosine methylation analysis. *Nucleic Acids Res.* 1996 Dec 15;24(24):5064-6). Using this method, it is possible to analyse individual cells, which illustrates the potential of the method. However, currently only individual regions of a length of up to approximately 3000 base pairs are analysed, a global analysis of cells for thousands of possible methylation events is not possible. However, this method cannot reliably analyse very small fragments from small sample quantities either. These are lost through the matrix in spite of the diffusion protection.

An overview of the further known methods of detecting 5-methylcytosine may be gathered from the following review article: Rein, T., DePamphilis, M. L., Zorbas, H., *Nucleic Acids Res.* 1998, 26, 2255.

To date, barring few exceptions (e.g., Zeschnigk M, Lich C, Buiting K, Doerfler W, Horsthemke B. A single-tube PCR test for the diagnosis of Angelman and Prader-Willi syndrome based on allelic methylation differences at the SNRPN locus. *Eur J Hum Genet.* 1997 Mar-Apr;5(2):94-8) the bisulfite technique is only used in research. Always, however, short, specific fragments of a known gene are amplified subsequent to a bisulfite treatment and either completely sequenced (Olek A, Walter J. The pre-implantation ontogeny of the H19 methylation imprint. *Nat Genet.* 1997 Nov;17(3):275-6) or individual cytosine positions are detected by a primer extension reaction (Gonzalzo ML, Jones PA. Rapid quantitation of methylation differences at specific sites using methylation-sensitive single nucleotide primer extension (Ms-SNuPE). *Nucleic Acids Res.* 1997 Jun 15;25(12):2529-31, WO Patent 9500669) or by enzymatic digestion (Xiong Z, Laird PW. COBRA: a sensitive and quantitative DNA methylation assay. *Nucleic Acids Res.* 1997 Jun 15;25(12):2532-4). In addition, detection by hybridisation has also been described (Olek et al., WO 99/28498).

Further publications dealing with the use of the bisulfite technique for methylation detection in individual genes are: Grigg G, Clark S. Sequencing 5-methylcytosine residues in genomic DNA. *Bioessays.* 1994 Jun;16(6):431-6, 431; Zeschnigk M, Schmitz B, Dittrich B, Buiting K, Horsthemke B, Doerfler W. Imprinted segments in the human genome: different DNA methylation patterns in the Prader-Willi/Angelman syndrome region as determined by the genomic sequencing method. *Hum Mol Genet.* 1997 Mar;6(3):387-95; Feil R, Charlton J, Bird AP,

MALDI-TOF spectrometry is excellently suited to the analysis of peptides and proteins. The analysis of nucleic acids is somewhat more difficult (Gut I G, Beck S. DNA and Matrix Assisted Laser Desorption Ionization Mass Spectrometry. Current Innovations and Future Trends. 1995, 1; 147-57). The sensitivity to nucleic acids is approximately 100 times worse than to peptides and decreases disproportionally with increasing fragment size. For nucleic acids having a multiply negatively charged backbone, the ionization process via the matrix is considerably less efficient. In MALDI-TOF spectrometry, the selection of the matrix plays an

eminently important role. For the desorption of peptides, several very efficient matrixes have been found which produce a very fine crystallization. There are now several responsive matrixes for DNA, however, the difference in sensitivity has not been reduced. The difference in sensitivity can be reduced by chemically modifying the DNA in such a manner that it becomes more similar to a peptide. Phosphorothioate nucleic acids in which the usual phosphates of the backbone are substituted with thiophosphates can be converted into a charge-neutral DNA using simple alkylation chemistry (Gut IG, Beck S. A procedure for selective DNA alkylation and detection by mass spectrometry. *Nucleic Acids Res.* 1995 Apr 25;23(8):1367-73). The coupling of a charge tag to this modified DNA results in an increase in sensitivity to the same level as that found for peptides. A further advantage of charge tagging is the increased stability of the analysis against impurities which make the detection of unmodified substrates considerably more difficult.

Genomic DNA is obtained from DNA of cell, tissue or other test samples using standard methods. This standard methodology is found in references such as Fritsch and Maniatis eds., *Molecular Cloning: A Laboratory Manual*, 1989.

Breast cancer is currently the second most common type of cancer amongst women. In 2001 over 190,000 new cases of invasive breast cancer and over 47, 000 additional cases of *in situ* breast cancer were diagnosed. Incidence and death rates increase with age, for the period 1994 - 1998 the incidence of breast cancer amongst women between the ages of 20 and 24 was only 1.5 per 100,000 population. However the risk increases to 489.7 within the age group 75 - 79. Mortality rates have decreased by approximately 5% over the last decade and factors affecting 5 year survival rates include age, stage of cancer, socioeconomic factors and race.

Breast cancer is defined as the uncontrolled proliferation of cells within breasts tissues. Breasts are comprised of 15 to 20 lobes joined together by ducts. Cancer arises most commonly in the duct, but is also found in the lobes with the rarest type of cancer termed inflammatory breast cancer.

It will be appreciated by those skilled in the art that there exists a continuing need to improve methods of early detection, classification and treatment of breast cancers. In contrast to the detection of some other common cancers such as cervical and dermal there are inherent difficulties in classifying and detecting breast cancers.

The first step of any treatment is the assessment of the patient's condition comparative to defined classifications of the disease. However the value of such a system is inherently dependant upon the quality of the classification. Breast cancers are staged according to their size, location and occurrence of metastasis. Methods of treatment include the use of surgery, radiation therapy, chemotherapy and hormone therapy, which are also used as adjuvant therapies to surgery.

Endocrine therapies have been developed in order to block the effects of estrogen on cancer cells or to reduce serum estrogen levels. Tamoxifen (TAM) is the most widely used antioestrogenic drug for breast cancer patients. It acts blocking estrogen stimulation of breast cancer cells, inhibiting both translocation and nuclear binding of estrogen receptor. TAM is used in adjuvant setting for primary breast cancer patients and for the treatment of metastatic disease and its effectiveness has been proved in several clinical trials. Treatment for five years reduces annual disease recurrence by 47% and annual deaths by 26% and this reduction is similar in different age groups. Also it reduces the incidence of developing contralateral breast tumours. TAM is among the least toxic antineoplastic agents but as it has estrogenic properties in some tissues, it increases by 3 folds the risk of endometrial cancer.

Hormone receptor status should be tested prior to TAM treatment as only a group of patients will benefit from therapy. For this purpose ER (estrogen receptor) and PR (progesterone receptor) status are tested routinely in all patients and they are considered predictive markers of response (a predictive marker or factor is any measurement associated with response or lack of response to a particular therapy). ER status is predictive of response in adjuvant TAM setting and also in advanced metastatic disease.

Only ER positive and or PR positive patients receive TAM and the remaining 10% of patients receive chemotherapy. The problem is that the treatment is only effective in a subgroup of hormone receptor positive patients and that a small subgroup of ER negative patients appear initially to respond to TAM. Then, the non responder subgroup will not only not benefit from TAM treatment but have the adverse effects of it and will not have the opportunity to receive chemotherapy instead. On the other hand, the ER negative subgroup that could have benefit from tamoxifen treatment will receive instead chemotherapy. Several different assays are available to measure PR and ER, including biochemical and IHC (immunohistochemical)

analysis, but there is a lack of standardisation of staining methods and interlaboratory variability (Clin Cancer Res 2000, 6:616-621).

Currently several predictive markers are under evaluation. Among them high bcl-2 levels showed promising correlation to TAM therapy response in patients with metastatic disease and prolonged survival and added valuable information to ER negative patient subgroup (J Clin Oncology 1997, 15 5:1916-1922; Endocrine, 2000, 13(1):1-10. There is conflicting evidence regarding the independent predictive value of c-erbB2 (Her2/neu) over expression in patients with advance breast cancer that require further evaluation and verification (British J of Cancer 1999, 79 (7/8):1220-1226; J Natl Cancer Inst, 1998,90 (21): 1601-1608).

Other predictive markers include SRC-1 (steroid receptor coactivator-1), CGA gene over expression, cell kinetics and S phase fraction assays (Breast Cancer Res and Treat , 1998, 48:87-92; Oncogene 2001, 20:6955-6959). Recently, uPA (Urokinase-type plasminogen activator) and PAI-1 (Plasminogen activator inhibitor type 1) together showed to be useful to define a subgroup of patients who have worse prognosis and who would benefit from adjuvant systemic therapy (J Clinical Oncology, 2002, 20 n° 4). All of these markers need further evaluations in prospective trials as none of them is yet a validated marker of response.

The gene MSMB (Accession number NM_002443) has been mapped to 10q11.2. It encodes the beta-microseminoprotein (MSP) which is one of the major proteins secreted by the prostate. Furthermore, it may be useful as a diagnostic marker for prostate cancer. Using mRNA analysis low levels of beta-MSP mRNA expression and protein have been linked to progression under endocrine therapy and it has been postulated that it may be indicative of potentially aggressive prostate cancer (see Sakai H, Tsurusaki T, Kanda S, Koji T, Xuan JW, Saito Y. 'Prognostic significance of beta-microseminoprotein mRNA expression in prostate cancer.' Prostate. 1999 Mar 1;38(4):278-84.).

The gene TP53 (Accession number NM_000546) encodes the protein p53, one of the most well characterised tumour suppressor proteins. The p53 protein acts as a transcription factor and serves as a key regulator of the cell cycle. Inactivation of this gene through mutation disrupts the cell cycle, which, in turn, assists in tumour formation. Methylation changes associated with this gene have been reported to be significant in breast cancer. Saraswati *et. al.* (Nature 405, 974 - 978 (22 Jun 2000) 'Compromised HOXA5 function can limit p53 expres-

sion in human breast tumours' reported that low levels of p53 mRNA in breast tumours was correlated to methylation of the HOXA5 gene. The product of the HOXA5 gene binds to the promoter region of the p53 and mediates expression of the gene. Methylation of the promoter region of the p53 gene itself has been reported (Kang JH, Kim SJ, Noh DY, Park IA, Choe KJ, Yoo OJ, Kang HS. 'Methylation in the p53 promoter is a supplementary route to breast carcinogenesis: correlation between CpG methylation in the p53 promoter and the mutation of the p53 gene in the progression from ductal carcinoma in situ to invasive ductal carcinoma,' Lab Invest. 2001 Apr;81(4):573-9.). It was therein demonstrated that CpG methylation in the p53 promoter region is found in breast cancer and it was hypothesised that methylation in the p53 promoter region could be an alternative pathway to neoplastic progression in breast tumors. It has been observed that treatment with Tamoxifen decreases the level of expression of the p53 gene (Farzadi E, Kaszas I, Baki M, Szende B. 'Changes in apoptosis, mitosis, Her-2, p53 and Bcl2 expression in breast carcinomas after short-term tamoxifen treatment.' Neoplasma. 2002;49(2):101-3.)

The gene CYP2D6 (Accession number: NM_000106) is a member of the human cytochrome P450 (CYP) superfamily. Many members of this family are involved in drug metabolism (see for example Curr Drug Metab. 2002 Jun;3(3):289-309. Rodrigues AD, Rushmore TH.), of these Cytochrome P450 CYP2D6 is one of the most extensively characterised. It is highly polymorphic (more than 70 variations of the gene have been described), and allelic variation can result in both increased and decreased enzymatic activity. The CYP2D6 enzyme catalyses the metabolism of a large number of clinically important drugs including antidepressants, neuroleptics, some antiarrhythmics (Nature 1990 Oct 25;347(6295):773-6 Identification of the primary gene defect at the cytochrome P450, CYP2D locus. Gough AC, Miles JS, Spurr NK, Moss JE, Gaedigk A, Eichelbaum M, Wolf CR.).

The gene PTGS2 (Accession number NM_000963) encodes an inducible isozyme of prostaglandin-endoperoxide synthase (prostaglandin-endoperoxide synthase 2). Aberrant methylation of this gene has been identified in lung carcinomas (Cancer Epidemiol Biomarkers Prev 2002 Mar;11(3):291-7 Hierarchical clustering of lung cancer cell lines using DNA methylation markers. Virmani AK, Tsou JA, Siegmund KD, Shen LY, Long TI, Laird PW, Gazdar AF, Laird-Offringa IA.).

The gene PSA (Accession number NM_021154), is not to be confused with the gene also popularly referred to as PSA (Accession number NM_001648) which encodes prostate specific antigen and whose technically correct name is Kallikrein 3. The gene PSA encodes the protein phosphoserine aminotransferase which is the second step-catalyzing enzyme in the serine biosynthesis pathway. Changes in gene expression levels have been monitored by mRNA expression analysis and upregulation of the gene has been identified in colonic carcinoma in a study of 6 samples (Electrophoresis 2002 Jun;23(11):1667-76 mRNA differential display of gene expression in colonic carcinoma. Ojala P, Sundstrom J, Gronroos JM, Virtanen E, Talvinen K, Nevalainen TJ.).

The gene CGA (Accession number NM_000735) encodes the alpha polypeptide of glycoprotein hormones. Further, it has been identified as an estrogen receptor alpha (ER alpha)-responsive gene and overexpression of the gene has been linked to ER positivity in breast tumours. Bieche *et. al.* examined mRNA levels of said gene in 125 ER alpha-positive postmenopausal breast cancer patients treated with primary surgery followed by adjuvant tamoxifen therapy. Initial results indicated significant links between CGA gene overexpression and Scarff-Bloom-Richardson histopathological grade I+II and progesterone and estrogen receptor positivity, which suggested that CGA is a marker of low tumour aggressiveness ('Identification of CGA as a Novel Estrogen Receptor-responsive Gene in Breast Cancer: An Outstanding Candidate Marker to predict the Response to Endocrine Therapy Cancer Research' 61, 1652-1658, February 15, 2001. Ivan Bièche, Béatrice Parfait, Vivianne Le Doussal, Martine Olivi, Marie-Christine Rio, Rosette Lidereau and Michel Vidaud). Further mRNA expression analysis linked CGA expression levels to Tamoxifen response, it was postulated that when combined with analysis of the marker ERBB2 (a marker of poor response) the gene may be useful as a predictive marker of tamoxifen responsiveness in breast cancer (Oncogene 2001 Oct 18;20(47):6955-9 The CGA gene as new predictor of the response to endocrine therapy in ER alpha-positive postmenopausal breast cancer patients. Bieche I, Parfait B, Nogues C, Andrieu C, Vidaud D, Spyrtos F, Lidereau R, Vidaud M.). The authors provided significant data associating the expression of the gene CGA with Tamoxifen treatment response. However, said analyses have all focused upon the analysis of relative levels of mRNA expression. This is not a methodology that is suitable for a medium or high throughput, nor is it a suitable basis for the development of a clinical assay.

The gene FGFR1 (Accession numbers NM_000604&NM_015850) encodes the heparin-binding growth factor receptor also known as the fibroblast growth factor receptor 1. It has been hypothesised that overexpression of FGFR1 seems to be associated with small, well-differentiated diploid tumours (Int J Cancer 1994 Nov 1;59(3):373-8 Expression of the FGFR1 gene in human breast-carcinoma cells. Jacquemier J, Adelaide J, Parc P, Penault-Llorca F, Planche J, deLapeyriere O, Birnbaum D.).

Description

The present invention provides methods and nucleic acids for evaluating the response of a patient with a cell proliferative disorder of the breast tissues to treatment with the drug Tamoxifen. Using the methods and nucleic acids described herein, statistically significant models of patient responsiveness to said treatment regimen can be developed and utilised to assist patients and clinicians in determining treatment options. The described method is to be used to assess the suitability of Tamoxifen as a therapy for patients suffering from a cell proliferative disorder of the breast tissues. Thus, the present invention will be seen to reduce the problems associated with present treatment response prediction methods.

Using the methods and nucleic acids as described herein, patient responsiveness can be evaluated before or during treatment with the drug Tamoxifen for a cell proliferative disorder of the breast tissues, in order to provide critical information to the patient and clinician as to the risks, burdens, and benefits associated with Tamoxifen treatment. It will be appreciated, therefore, that the methods and nucleic acids exemplified herein can serve to improve a patient's quality of life and odds of treatment success by allowing both patient and clinician a more accurate assessment of the patient's treatment options.

The aim of the invention is achieved by means of the analysis of the methylation patterns of one or a combination of genes taken from the group FGFR1, PSA, CGA, PTGS2, MSMB, TP53 and CYP2D6 (see Table 1) and/or their regulatory regions. The invention is characterised in that the nucleic acid of one or a combination of genes taken from the group FGFR1, PSA, CGA, PTGS2, MSMB, TP53 and CYP2D6 is contacted with a reagent or series of reagents capable of distinguishing between methylated and non methylated CpG dinucleotides within the genomic sequence of interest.

The present invention makes available a method for ascertaining genetic and/or epigenetic parameters of genomic DNA. The method is for use in the improved treatment and monitoring of breast cell proliferative disorders, by enabling the accurate prediction of a patient's response to treatment with the drug Tamoxifen. In a particularly preferred embodiment, the method according to the invention enables the differentiation between patients who respond to therapy with the drug Tamoxifen and those who do not.

The method according to the invention may be used for the analysis of a wide variety of cell proliferative disorders of the breast tissues including, but not limited to ductal carcinoma *in situ*, lobular carcinoma, colloid carcinoma, tubular carcinoma, medullary carcinoma, metastatic carcinoma, intraductal carcinoma *in situ*, lobular carcinoma *in situ* and papillary carcinoma *in situ*.

The method according to the invention is particularly suited to the prediction of response to Tamoxifen treatment in the two main applications of Tamoxifen. In one embodiment, the method is applied to patients who receive Tamoxifen treatment in an adjuvant setting (i.e. as secondary treatment to a primary treatment such as surgery), as illustrated in Figure 1. Such a treatment is often prescribed to patients suffering from Stage 1 to 3 carcinomas. In this embodiment responders are defined as those who do not have a detectable relapse of the breast cancer in a specified period of time, non responders are those who relapse within said time period. In this embodiment it is preferred that the target nucleic acids are one or a combination of genes taken from the group PTGS2, MSMB, TP53 and CYP2D6 and/or their regulatory regions. In this embodiment of the method it is preferred that the method according to the invention is applied only to patients who are estrogen and/or progesterone receptor markers.

In a further embodiment said method is applied to patients suffering from a metastatic cancer wherein Tamoxifen is the primary treatment, as illustrated in Figure 2. Such a treatment is often prescribed to patients suffering from later stage carcinomas, particularly wherein metastasis has occurred. In this embodiment it is preferred that the target nucleic acids are one or a combination of genes taken from the group PTGS2, MSMB, TP53 and CYP2D6 and/or their regulatory regions. In this embodiment responders are those who enter partial or complete remission i.e. subjects whose cancer recedes to undetectable levels as opposed to those whose diseases further metastasize or remain above detectable levels. This methodology presents further improvements over the state of the art in that the method may be applied to any subject, independent of the estrogen and/or progesterone receptor status. Therefore in a pre-

ferred embodiment, the subject is not required to have been tested for estrogen or progesterone receptor status. In a particularly preferred embodiment of the method the methodology is applied to subjects who have not received chemotherapy.

Furthermore, the method enables the analysis of cytosine methylations and single nucleotide polymorphisms within said gene.

The object of the invention is achieved by means of the analysis of the methylation patterns of the genes FGFR1, PSA, CGA, PTGS2, MSMB, TP53 and CYP2D6 and/or their regulatory regions, in a particularly preferred embodiment the sequences comprising essentially of SEQ ID NOS: 15, 31, 32, 63, 40, 9 and 33 and sequences complementary thereto. In a preferred embodiment said method is achieved by contacting said nucleic acid sequences in a biological sample obtained from a subject with at least one reagent or a series of reagents, wherein said reagent or series of reagents, distinguishes between methylated and non methylated CpG dinucleotides within the target nucleic acid.

In a preferred embodiment, the method comprises the following steps:

In the first step of the method the genomic DNA sample must be isolated from sources such as cells or cellular components which contain DNA, sources of DNA comprising, for example, cell lines, histological slides, biopsies, tissue embedded in paraffin, breast tissues, blood, plasma, lymphatic fluid, lymphatic tissue, duct cells, ductal lavage fluid, nipple aspiration fluid, bone marrow and combinations thereof. Extraction may be by means that are standard to one skilled in the art, these include the use of detergent lysates, sonification and vortexing with glass beads. Once the nucleic acids have been extracted the genomic double stranded DNA is used in the analysis.

In a preferred embodiment the DNA may be cleaved prior to the next step of the method, this may be by any means standard in the state of the art, in particular, but not limited to, with restriction endonucleases.

In the second step of the method, the genomic DNA sample is treated in such a manner that cytosine bases which are unmethylated at the 5'-position are converted to uracil, thymine, or another base which is dissimilar to cytosine in terms of hybridisation behaviour. This will be understood as 'pretreatment' hereinafter.

The above described treatment of genomic DNA is preferably carried out with bisulfite (sulfite, disulfite) and subsequent alkaline hydrolysis, which results in a conversion of non-methylated cytosine nucleobases to uracil or to another base which is dissimilar to cytosine in terms of base pairing behaviour. If bisulfite solution is used for the reaction, then an addition takes place at the non-methylated cytosine bases. Moreover, a denaturing reagent or solvent as well as a radical interceptor must be present. A subsequent alkaline hydrolysis then gives rise to the conversion of non-methylated cytosine nucleobases to uracil. The converted DNA is then used for the detection of methylated cytosines.

Fragments of the pretreated DNA are amplified, using sets of primer oligonucleotides, and a preferably heat-stable, polymerase. Because of statistical and practical considerations, preferably more than ten different fragments having a length of 100 - 2000 base pairs are amplified. The amplification of several DNA segments can be carried out simultaneously in one and the same reaction vessel. Usually, the amplification is carried out by means of a polymerase chain reaction (PCR).

The design of such primers is obvious to one skilled in the art. These should include at least two oligonucleotides whose sequences are each reverse complementary or identical to an at least 18 base-pair long segment of the following base sequences specified in the appendix SEQ ID NOS: SEQ ID NOS: 15, 31, 32, 63, 40, 9, 33, 109, 110, 234, 235, 141, 142, 267, 268, 143, 144, 269, 270, 205, 206, 331, 332, 159, 160, 285, 286, 97, 98, 223, 224, 145, 146, 271 and 272. Said primer oligonucleotides are preferably characterised in that they do not contain any CpG dinucleotides. In a particularly preferred embodiment of the method, the sequence of said primer oligonucleotides are designed so as to selectively anneal to and amplify, only the breast cell specific DNA of interest, thereby minimising the amplification of background or non relevant DNA. In the context of the present invention, background DNA is taken to mean genomic DNA which does not have a relevant tissue specific methylation pattern, in this case, the relevant tissue being breast tissues.

According to the present invention, it is preferred that at least one primer oligonucleotide is bound to a solid phase during amplification. The different oligonucleotide and/or PNA-oligomer sequences can be arranged on a plane solid phase in the form of a rectangular or hexagonal lattice, the solid phase surface preferably being composed of silicon, glass, poly-

styrene, aluminium, steel, iron, copper, nickel, silver, or gold, it being possible for other materials such as nitrocellulose or plastics to be used as well.

The fragments obtained by means of the amplification may carry a directly or indirectly detectable label. Preferred are labels in the form of fluorescence labels, radionuclides, or detachable molecule fragments having a typical mass which can be detected in a mass spectrometer, it being preferred that the fragments that are produced have a single positive or negative net charge for better detectability in the mass spectrometer. The detection may be carried out and visualised by means of matrix assisted laser desorption/ionisation mass spectrometry (MALDI) or using electron spray mass spectrometry (ESI).

In the next step the nucleic acid amplicates are analysed in order to determine the methylation status of the genomic DNA prior to treatment.

The post treatment analysis of the nucleic acids may be carried out using alternative methods. Several methods for the methylation status specific analysis of the treated nucleic acids are described below, other alternative methods will be obvious to one skilled in the art.

Using several methods known in the art the analysis may be carried out during the amplification step of the method. In one such embodiment, the methylation status of preselected CpG positions within the genes FGFR1, PSA, CGA, PTGS2, MSMB, TP53 and CYP2D6 and/or their regulatory regions may be detected by use of methylation specific primer oligonucleotides. This technique has been described in U.S. Patent 6,265,171 to Herman. The use of methylation status specific primers for the amplification of bisulphite treated DNA allows the differentiation between methylated and unmethylated nucleic acids. MSP primers pairs contain at least one primer which hybridises to a bisulphite treated CpG dinucleotide. Therefore the sequence of said primers comprises at least one CG, TG or CA dinucleotide. MSP primers specific for non methylated DNA contain a 'T' at the 3' position of the C position in the CpG. According to the present invention, it is therefore preferred that the base sequence of said primers is required to comprise a sequence having a length of at least 9 nucleotides which hybridises to a pretreated nucleic acid sequence according to SEQ ID NOS: 109, 110, 234, 235, 141, 142 267, 268, 143, 144, 269, 270, 205, 206, 331, 332, 159, 160, 285, 286, 97, 98, 223, 224, 145, 146, 271 or 272 and sequences complementary thereto wherein the base sequence of said oligomers comprises at least one CG, TG or CA dinucleotide.

In one embodiment of the method the methylation status of the CpG positions may be determined by means of hybridisation analysis. In this embodiment of the method the amplicates obtained in the second step of the method are hybridised to an array or a set of oligonucleotides and/or PNA probes. In this context, the hybridisation takes place in the manner described as follows. The set of probes used during the hybridisation is preferably composed of at least 4 oligonucleotides or PNA-oligomers. In the process, the amplicates serve as probes which hybridise to oligonucleotides previously bonded to a solid phase. The non-hybridised fragments are subsequently removed. Said oligonucleotides contain at least one base sequence having a length of 10 nucleotides which is reverse complementary or identical to a segment of the base sequences specified in the appendix, the segment containing at least one CpG or TpG dinucleotide. In a further preferred embodiment the cytosine of the CpG dinucleotide, or in the case of TpG, the thiamine, is the 5th to 9th nucleotide from the 5'-end of the 10-mer. One oligonucleotide exists for each CpG or TpG dinucleotide.

The non-hybridised amplicates are then removed. In the final step of the method, the hybridised amplicates are detected. In this context, it is preferred that labels attached to the amplicates are identifiable at each position of the solid phase at which an oligonucleotide sequence is located.

In a further embodiment of the method the methylation status of the CpG positions may be determined by means of oligonucleotide probes that are hybridised to the treated DNA concurrently with the PCR amplification primers (wherein said primers may either be methylation specific or standard).

A particularly preferred embodiment of this method is the use of fluorescence-based Real Time Quantitative PCR (Heid et al., Genome Res. 6:986-994, 1996) employing a dual-labelled fluorescent oligonucleotide probe (TaqMan™ PCR, using an ABI Prism 7700 Sequence Detection System, Perkin Elmer Applied Biosystems, Foster City, California). The TaqMan™ PCR reaction employs the use of a nonextendible interrogating oligonucleotide, called a TaqMan™ probe, which is designed to hybridize to a GpC-rich sequence located between the forward and reverse amplification primers. The TaqMan™ probe further comprises a fluorescent "reporter moiety" and a "quencher moiety" covalently bound to linker moieties (e.g., phosphoramidites) attached to the nucleotides of the TaqMan™ oligonucleo-

tide. For analysis of methylation within nucleic acids subsequent to bisulphite treatment it is required that the probe be methylation specific, as described in U.S. 6,331,393, (hereby incorporated by reference) also known as the Methyl Light assay. Variations on the TaqMan™ detection methodology that are also suitable for use with the described invention include the use of dual probe technology (Lightcycler™) or fluorescent amplification primers (Sunrise™ technology). Both these techniques may be adapted in a manner suitable for use with bisulphite treated DNA, and moreover for methylation analysis within CpG dinucleotides.

A further suitable method for the use of probe oligonucleotides for the assessment of methylation by analysis of bisulphite treated nucleic acids is the use of blocker oligonucleotides. The use of such oligonucleotides has been described in BioTechniques 23(4), 1997, 714-720 D. Yu, M. Mukai, Q. Liu, C. Steinman. Blocking probe oligonucleotides are hybridised to the bisulphite treated nucleic acid concurrently with the PCR primers. PCR amplification of the nucleic acid is terminated at the 5' position of the blocking probe, thereby amplification of a nucleic acid is suppressed wherein the complementary sequence to the blocking probe is present. The probes may be designed to hybridise to the bisulphite treated nucleic acid in a methylation status specific manner. For example, for detection of methylated nucleic acids within a population of unmethylated nucleic acids suppression of the amplification of nucleic acids which are unmethylated at the position in question would be carried out by the use of blocking probes comprising a 'CG' at the position in question, as opposed to a 'CA'.

In a further preferred embodiment of the method the determination of the methylation status of the CpG positions is carried out by the use of template directed oligonucleotide extension, such as MS SNUPE as described by Gonzalgo and Jones (Nucleic Acids Res. 25:2529-2531).

In a further embodiment of the method the determination of the methylation status of the CpG positions is enabled by sequencing and subsequent sequence analysis of the amplificate generated in the second step of the method (Sanger F., et al., 1977 PNAS USA 74: 5463-5467).

A further embodiment of the invention is a method for the analysis of the methylation status of genomic DNA without the need for pretreatment. In the first and second steps of the method the genomic DNA sample must be obtained and isolated from tissue or cellular sources. Such sources may include cell lines, histological slides, body fluids, or tissue embedded in paraffin. Extraction may be by means that are standard to one skilled in the art, these

include the use of detergent lysates, sonification and vortexing with glass beads. Once the nucleic acids have been extracted the genomic double stranded DNA is used in the analysis.

In a preferred embodiment the DNA may be cleaved prior to the treatment, this may be by any means standard in the state of the art, in particular with restriction endonucleases. In the third step, the DNA is then digested with one or more methylation sensitive restriction enzymes. The digestion is carried out such that hydrolysis of the DNA at the restriction site is informative of the methylation status of a specific CpG dinucleotide.

In a preferred embodiment the restriction fragments are amplified. In a further preferred embodiment this is carried out using the polymerase chain reaction.

In the final step the amplicates are detected. The detection may be by any means standard in the art, for example, but not limited to, gel electrophoresis analysis, hybridisation analysis, incorporation of detectable tags within the PCR products, DNA array analysis, MALDI or ESI analysis.

The aforementioned method is preferably used for ascertaining genetic and/or epigenetic parameters of genomic DNA.

In order to enable this method, the invention further provides the modified DNA of one or a combination of genes taken from the group FGFR1, PSA, CGA, PTGS2, MSMB, TP53 and CYP2D6 as well as oligonucleotides and/or PNA-oligomers for detecting cytosine methylations within said genes. The present invention is based on the discovery that genetic and epigenetic parameters and, in particular, the cytosine methylation patterns of said genomic DNAs are particularly suitable for improved treatment and monitoring of breast cell proliferative disorders.

The nucleic acids according to the present invention can be used for the analysis of genetic and/or epigenetic parameters of genomic DNA.

This objective according to the present invention is achieved using a nucleic acid containing a sequence of at least 18 bases in length of the pretreated genomic DNA according to one of SEQ ID NOS: 109, 110, 234, 235, 141, 142 267, 268, 143, 144, 269, 270, 205, 206, 331, 332,

159, 160, 285, 286, 97, 98, 223, 224, 145, 146, 271 and 272 and sequences complementary thereto.

The modified nucleic acids could heretofore not be connected with the ascertainment of disease relevant genetic and epigenetic parameters.

The object of the present invention is further achieved by an oligonucleotide or oligomer for the analysis of pretreated DNA, for detecting the genomic cytosine methylation state, said oligonucleotide containing at least one base sequence having a length of at least 10 nucleotides which hybridises to a pretreated genomic DNA according to SEQ ID NOS: 109, 110, 234, 235, 141, 142 267, 268, 143, 144, 269, 270, 205, 206, 331, 332, 159, 160, 285, 286, 97, 98, 223, 224, 145, 146, 271 and 272. The oligomer probes according to the present invention constitute important and effective tools which, for the first time, make it possible to ascertain specific genetic and epigenetic parameters during the analysis of biological samples for features associated with a patient's response to Tamoxifen treatment. Said oligonucleotides allow the improved treatment and monitoring of breast cell proliferative disorders. The base sequence of the oligomers preferably contains at least one CpG or TpG dinucleotide. The probes may also exist in the form of a PNA (peptide nucleic acid) which has particularly preferred pairing properties. Particularly preferred are oligonucleotides according to the present invention in which the cytosine of the CpG dinucleotide is within the middle third of said oligonucleotide e.g. the 5th - 9th nucleotide from the 5'-end of a 13-mer oligonucleotide; or in the case of PNA-oligomers, it is preferred for the cytosine of the CpG dinucleotide to be the 5th - 6th nucleotide from the 5'-end of the 9-mer.

The oligomers according to the present invention are normally used in so called "sets" which contain at least one oligomer for each of the CpG dinucleotides within SEQ ID NOS: 109, 110, 234, 235, 141, 142 267, 268, 143, 144, 269, 270, 205, 206, 331, 332, 159, 160, 285, 286, 97, 98, 223, 224, 145, 146, 271 and 272.

In a particularly preferred embodiment of the method the oligomers according to SEQ ID NOS: 955, 955, 956, 956, 957, 957, 958, 958 - 964 are used for predicting a subject's response to Tamoxifen treatment in an adjuvant setting. In a further particularly preferred embodiment of the method the oligomers according to SEQ ID NOS: 941 - 954 are used for predicting a subject's response to Tamoxifen treatment in an metastatic setting.

In the case of the sets of oligonucleotides according to the present invention, it is preferred that at least one oligonucleotide is bound to a solid phase. It is further preferred that all the oligonucleotides of one set are bound to a solid phase.

The present invention further relates to a set of at least 10 n (oligonucleotides and/or PNA-oligomers) used for detecting the cytosine methylation state of genomic DNA, by analysis of said sequence or treated versions of said sequence (of the genes FGFR1, PSA, CGA, PTGS2, MSMB, TP53 and CYP2D6, as detailed in the sequence listing and Table 1) and sequences complementary thereto). These probes enable improved treatment and monitoring of breast cell proliferative disorders.

The set of oligomers may also be used for detecting single nucleotide polymorphisms (SNPs) by analysis of said sequence or treated versions of said sequence (of the genes FGFR1, PSA, CGA, PTGS2, MSMB, TP53 and CYP2D6, as detailed in the sequence listing and Table 1).

It will be obvious to one skilled in the art that the method according to the invention will be improved and supplemented by the incorporation of markers and clinical indicators known in the state of the art and currently used as predictive of the outcome Tamoxifen treatment. More preferably said markers include node status, age, menopausal status, grade, estrogen and progesterone receptors.

The genes that form the basis of the present invention may also be used to form a "gene panel", i.e. a collection comprising the particular genetic sequences of the present invention and/or their respective informative methylation sites. The formation of gene panels allows for a quick and specific analysis of specific aspects of breast cancer treatment. The gene panel(s) as described and employed in this invention can be used with surprisingly high efficiency for the treatment of breast cell proliferative disorders by prediction of the outcome of treatment with the drug Tamoxifen.

The efficiency of the method according to the invention is improved when applied to patients who have not been treated with chemotherapy. Accordingly, it is a particularly preferred embodiment of the method wherein the method is used for the assessment of subjects who have not undergone chemotherapy.

In addition, the use of multiple CpG sites from a diverse array of genes allows for a relatively high degree of sensitivity and specificity in comparison to single gene prognostic tools.

According to the present invention, it is preferred that an arrangement of different oligonucleotides and/or PNA-oligomers (a so-called "array") made available by the present invention is present in a manner that it is likewise bound to a solid phase. This array of different oligonucleotide- and/or PNA-oligomer sequences can be characterised in that it is arranged on the solid phase in the form of a rectangular or hexagonal lattice. The solid phase surface is preferably composed of silicon, glass, polystyrene, aluminium, steel, iron, copper, nickel, silver, or gold. However, nitrocellulose as well as plastics such as nylon which can exist in the form of pellets or also as resin matrices are suitable alternatives.

Therefore, a further subject matter of the present invention is a method for manufacturing an array fixed to a carrier material for the improved treatment and monitoring of breast cell proliferative disorders. In said method at least one oligomer according to the present invention is coupled to a solid phase. Methods for manufacturing such arrays are known, for example, from US Patent 5,744,305 by means of solid-phase chemistry and photolabile protecting groups.

A further subject matter of the present invention relates to a DNA chip for the improved treatment and monitoring of breast cell proliferative disorders. The DNA chip contains at least one nucleic acid according to the present invention. DNA chips are known, for example, in US Patent 5,837,832.

Moreover, a subject matter of the present invention is a kit which may be composed, for example, of a bisulfite-containing reagent, a set of primer oligonucleotides containing at least two oligonucleotides whose sequences in each case correspond to or are complementary to a 18 base long segment of the base sequences specified in SEQ ID NOS: 109, 110, 234, 235, 141, 142 267, 268, 143, 144, 269, 270, 205, 206, 331, 332, 159, 160, 285, 286, 97, 98, 223, 224, 145, 146, 271 and 272, oligonucleotides and/or PNA-oligomers as well as instructions for carrying out and evaluating the described method.

In a further preferred embodiment said kit may further comprise standard reagents for performing a CpG position specific methylation analysis wherein said analysis comprises one or more of the following techniques: MS-SNuPE, MSP, Methyl light, Heavy Methyl, and nucleic acid sequencing. However, a kit along the lines of the present invention can also contain only part of the aforementioned components.

The oligomers according to the present invention or arrays thereof as well as a kit according to the present invention are intended to be used for the improved treatment and monitoring of breast cell proliferative disorders. According to the present invention, the method is preferably used for the analysis of important genetic and/or epigenetic parameters within genomic DNA, in particular for use in improved treatment and monitoring of breast cell proliferative disorders.

The methods according to the present invention are used, for improved treatment and monitoring of breast cell proliferative disorder by enabling more informed treatment regimens.

The present invention moreover relates to the diagnosis and/or prognosis of events which are disadvantageous or relevant to patients or individuals in which important genetic and/or epigenetic parameters within genomic DNA, said parameters obtained by means of the present invention may be compared to another set of genetic and/or epigenetic parameters, the differences serving as the basis for the diagnosis and/or prognosis of events which are disadvantageous or relevant to patients or individuals. Examples of such parameters include, but are not limited to the assessment of estrogen and progesterone markers.

In the context of the present invention the term "hybridisation" is to be understood as a bond of an oligonucleotide to a completely complementary sequence along the lines of the Watson-Crick base pairings in the sample DNA, forming a duplex structure.

In the context of the present invention, "genetic parameters" are mutations and polymorphisms of genomic DNA and sequences further required for their regulation. To be designated as mutations are, in particular, insertions, deletions, point mutations, inversions and polymorphisms and, particularly preferred, SNPs (single nucleotide polymorphisms).

In the context of the present invention the term „methylation state“ is taken to mean the degree of methylation present in a nucleic acid of interest, this may be expressed in absolute or relative terms i.e. as a percentage or other numerical value or by comparison to another tissue and therein described as hypermethylated, hypomethylated or as having significantly similar or identical methylation status.

In the context of the present invention the term „regulatory region“ of a gene is taken to mean nucleotide sequences which affect the expression of a gene. Said regulatory regions may be located within, proximal or distal to said gene. Said regulatory regions include but are not limited to constitutive promoters, tissue-specific promoters, developmental-specific promoters, inducible promoters and the like. Promoter regulatory elements may also include certain enhancer sequence elements that control transcriptional or translational efficiency of the gene.

In the context of the present invention the term “chemotherapy” is taken to mean the use of drugs or chemical substances to treat cancer. This definition excludes radiation therapy (treatment with high energy rays or particles), hormone therapy (treatment with hormones or hormone analogs (synthetic substitutes) and surgical treatment.

In the context of the present invention, “epigenetic parameters“ are, in particular, cytosine methylations and further modifications of DNA bases of genomic DNA and sequences further required for their regulation. Further epigenetic parameters include, for example, the acetylation of histones which, cannot be directly analysed using the described method but which, in turn, correlates with the DNA methylation.

In the context of the present invention the term “adjuvant treatment” is taken to mean a therapy of a cancer patient immediately following an initial non chemotherapeutical therapy, e.g. surgery. In the context of the present invention, said adjuvant treatment is performed using Tamoxifen. In general, the purpose of an adjuvant therapy is to provide a significantly smaller risk of recurrences compared without the adjuvant therapy.

In the context of the present invention the term “estrogen and/or progesterone receptor positive” is taken to mean cells that expresses on its surface receptors that are susceptible to the binding of estrogens and/or progesterones.

In the following, the present invention will be explained in greater detail on the basis of the sequences, figures and examples without being limited thereto.

Figure 1 shows a preferred application of the method according to the invention. The X axis shows the tumour(s) mass, wherein the line '2' shows the limit of detectability. The Y-axis shows time. Accordingly said figure illustrates a simplified model of tamoxifen treatment of an Stage 1-3 breast tumour wherein primary treatment was surgery (at point 1), followed by adjuvant therapy with Tamoxifen. In a first scenario a responder to treatment (4) is shown as remaining below the limit of detectability for the duration of the observation. A non responder to the treatment (5) has a period of disease free survival (2) followed by relapse when the carcinoma mass reaches the level of detectability.

Figure 2 shows another preferred application of the method according to the invention. The X axis shows the tumour(s) mass, wherein the line '2' shows the limit of detectability. The Y-axis shows time. Accordingly said figure illustrates a simplified model of Tamoxifen treatment of an late stage breast tumour wherein primary treatment was surgery (at point 1), followed by relapse which is treated by Tamoxifen (2). In a first scenario a responder to treatment (4) is shown as remaining below the limit of detectability for the duration of the observation. A non responder to the treatment (5) does not recover from the relapse.

Figures 3 to 10 show a ranked matrix of additional data obtained according to Example 4 according to CpG methylation differences between the two classes of tissues, using an algorithm. Figures 3,5,7 and 9 are shown in greyscale, wherein the most significant CpG positions are at the bottom of the matrix with significance decreasing towards the top. Black indicates total methylation at a given CpG position, white represents no methylation at the particular position, with degrees of methylation represented in grey, from light (low proportion of methylation) to dark (high proportion of methylation). Each row represents one specific CpG position within a gene and each column shows the methylation profile for the different CpGs for one sample. On the left side a CpG and gene identifier is shown this may be cross referenced with the accompanying table (Table 3) in order to ascertain the gene in question and the detection oligomer used. On the right side p values for the individual CpG positions are shown. The p values are the probabilities that the observed distribution occurred by chance in the data set. Figures 4,6,8 and 10 are the red-green versions of the preceding figures (i.e. figures 3,5,7

and 9 respectively). red indicates total methylation at a given CpG position, green represents no methylation at the particular position.

SEQ ID NOS: 15, 31, 32, 63, 40, 9 and 33 represent 5' and/or regulatory regions and/or CpG rich regions of the genes according to Table 1. These sequences are derived from Genbank and will be taken to include all minor variations of the sequence material which are currently unforeseen, for example, but not limited to, minor deletions and SNPs.

SEQ ID NOS: 109, 110, 234, 235, 141, 142, 267, 268, 143, 144, 269, 270, 205, 206, 331, 332, 159, 160, 285, 286, 97, 98, 223, 224, 145, 146, 271 and 272 exhibit the pretreated sequence of DNA derived from the genomic sequence according to Table 1. These sequences will be taken to include all minor variations of the sequence material which are currently unforeseen, for example, but not limited to, minor deletions and SNPs.

SEQ ID NO: 459 to SEQ ID NO: 964 exhibit the sequence of oligomers which are useful for the analysis of CpG positions within genomic DNA according to SEQ ID NO: 1 to SEQ ID NO: 63 according to Tables 2 and 3.

SEQ ID NO: 941 to SEQ ID NO: 964 exhibit the sequence of oligomers which are particularly useful for the analysis of the methylation status of CpG positions of genomic DNA according to SEQ ID NOS: 15, 31, 32, 63, 40, 9 and 33.

Table 1: Informative marker genes

<i>Gene Name</i>	<i>Accession Number</i>	<i>Genomic sequence (SEQ ID NO:)</i>	<i>Treated sequences (SEQ ID NO:)</i>	<i>Treated sequence amplification primers (SEQ ID NO:)</i>	<i>Treated sequence hybridization oligonucleotides (SEQ ID NO:)</i>
FGFR1	NM_000604	15	109, 110, 234, 235	361-362	577-584, 941-944,
PSA	NM_021154	31	141, 142, 267, 268	393-394	701-704, 945-950.
CGA	NM_000735	32	143, 144, 269, 270	395-396	705-708, 952-954
PTGS2	NM_000963	63	205, 206, 331, 332	457-458	937-940, 955-958.
MSMB	NM_002443	40	159, 160, 285, 286.	411,41	763-766, 959, 960.
TP53	NM_000546	9	97, 98, 223, 224.	349,350.	531-536,

<i>Gene Name</i>	<i>Accession Number</i>	<i>Genomic sequence (SEQ ID NO:)</i>	<i>Treated sequences (SEQ ID NO:)</i>	<i>Treated sequence amplification primers (SEQ ID NO:)</i>	<i>Treated sequence hybridization oligonucleotides (SEQ ID NO:)</i>
					961,962.
CYP2D6	NM_000106	33	145, 146, 271, 272.	397, 398.	709-714, 963-964.

Examples

Example 1

The gene CYP2D6 was identified as being differentially hypermethylated in Tamoxifen non-responders as compared to Tamoxifen responders using the technique Methylated CpG Island Amplification (MCA). This method identifies hypermethylated sequences in one population of genomic DNA as compared to a second population by selectively eliminating sequences that do not contain the hypermethylated regions. This is done by digestion of genomic DNA with a methylation-sensitive enzyme that cleaves un-methylated restriction sites to leave blunt ends, followed by cleavage with an isoschizomer that is methylation insensitive and leaves sticky ends. This is followed by amplicon generation and subtractive hybridization of the tester population with the driver population.

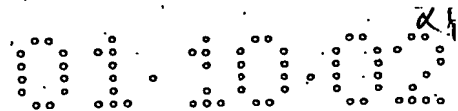
All samples came from patients treated with Tamoxifen as an adjuvant therapy immediately following surgery, as illustrated in Figure 1.

This MeST was found in only one comparison: Tamoxifen Responder pool vs. Tamoxifen Non-responder pool and was hypermethylated in the non-responder pool. Responder pools consisted of 16 samples (1.5 µg each, total of 24 µg). Non-Responder pools were 8 samples (3 µg each, total of 24 µg). Responder pools were Drivers, Non-responders were testers.

In the initial reactions 5 µg of each genomic DNA pool were digested with SmaI in a 100 µl reaction overnight at 25 degrees C in NEB buffer 4 + BSA, and 100 units of enzyme (10uL).

The pools were then further digested with Xma I (2 µl = 100 U), 6 hours at 37 degrees C.

500 ng of the cleaned-up, digested material was ligated to the adapter-primer SEQ ID NO:965 + SEQ ID NO:966 (Sequence: SEQ ID NO:965: AGCACTCTCCAGCCTCTCACCGAC; SEQ ID NO:966: CCGGGTCGGTGA. These were hybridised to create the adapter by heating together at 70 degrees C and slowly cooling to RT) in a 30 µl reaction overnight at 16 degrees, with 400 U (1 uL) of T4 ligase enzyme. 3 µl of the ligation mix for both tester and driver populations was used in each initial PCR to generate the starting amplicons. 2 PCR reactions were done for the tester and 8 for the driver. Reactions were 100 µl, with 1µl of 100 µM primer SEQ ID NO:965 (AGCACTCTCCAGCCTCTCACCGAC), 10 µl PCR buffer, 1.2 µl 25 mM dNTPs, 68.8 uL water, 1 µl titanium Taq, 2 µl DMSO, and 10 µl 5M Betaine. An



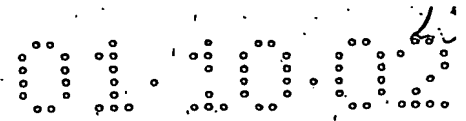
initial 95 degrees for 1 min step, followed by 25 cycles of 95 degrees for 1 min followed by 72 degrees for 3 min, and a final extension at 72 degrees for 10 min were done.

The tester amplicons were then digested with XmaI as described above, yielding overhanging ends and the driver amplicons were digested with SmaI as above, yielding blunt end fragments.

A new set of adapter primers (hybridised as described for RXMA primers) SEQ ID NO:967 + SEQ ID NO:968 (SEQ ID NO:967: ACCGACGTCGACTATCCATGAACC; SEQ ID NO:968: CCGGGGTTCATG) was ligated to the Tester only, using the same conditions as previously.

Five µg of digested tester and 40 µg of digested driver amplicons were hybridised in a solution containing 4 µl EE (30 mM EPPS, 3 mM EDTA) and 1 µl 5 M NaCl at 67 degrees C for 20 hours. A selective PCR reaction was done using primer SEQ ID NO:967 (Sequence: SEQ ID NO:967: ACCGACGTCGACTATCCATGAACC). The amplification was done as follows: An initial fill-in step of 72 degrees 5 min, followed by 95 degrees for 1 min, 72 degrees for 3 min for 10 cycles. 10 µl of Mung Bean nuclease buffer plus 10 µl Mung Bean Nuclease (10U) was added and incubated at 30 degrees for 30 min. This reaction was cleaned up and used as a template for 25 more cycles of PCR using SEQ ID NO:967 primer and the same conditions.

The resulting PCR product (tester) is then digested again using XmaI, as above, and a third adapter, SEQ ID NO:969 + SEQ ID NO:970 (Sequence: SEQ ID NO:969: AGGCAACTGTGCTATCCGAGTGAC; SEQ ID NO:970: CCGGGTCACTCG) was ligated. The tester (500 ng) was then hybridised a second time to the original digested driver (40 µg) in 4 µl EE (30 mM EPPS, 3 mM EDTA) and 1 µl 5 M NaCl at 67 degrees C for 20 hours. Selective PCR was performed using SEQ ID NO:969 primer (Sequence: SEQ ID NO:969: AGGCAACTGTGCTATCCGAGTGAC) as follows: an initial fill-in step of 72 degrees 5 min, followed by 95 degrees for 1 min, 72 degrees for 3 min for 10 cycles. 10 µl of Mung Bean nuclease buffer plus 10 µl Mung Bean Nuclease (10U) was added and incubated at 30 degrees for 30 min. This reaction was cleaned up and used as a template for 25 more cycles of PCR using SEQ ID NO:969 primer and the same conditions.



The resulting PCR product (1.8 μ g) was digested with XmaI (in 50 μ l total volume, NEB buffer 4 + BSA, and 2 μ l = 100 U XmaI, 6 hours at 37 degrees C.) and ligated into the vector pBC Sk- predigested with XmaI and phosphatased (675 ng). 5 μ l of a 30 μ l ligation was used to transform chemically competent TOP10 cells according to the manufacturer's instructions. The transformations were plated onto LB/XGal/IPTG/CAM plates. Approximately 50 insert-containing colonies, apparent due to their lack of blue colouring, were selected for sequencing.

The resulting insert positive clones were sequenced using vector primers and the sequence was compared to the Ensembl Golden Path genome database using the NCBI BLAST utility (ref.) housed on an internal server.

Examples 2 and 3

In the following examples, multiplex PCR of a large selection of genes according to Table 2 was carried out on tissue samples from breast cancer patients treated with the drug Tamoxifen, comparisons of responders to the drug to non responders to the drug in both the adjuvant and metastatic settings (See figures 1 and 2) was then carried out.

Each sample was treated in the manner described below in Example 2 in order to deduce the methylation status of CpG positions, the CpG methylation information for each sample was collated and then used in an analysis, as detailed in Example 3.

Example 2

In the first step the genomic DNA was isolated from the cell samples using the Wizzard kit from (Promega).

The isolated genomic DNA from the samples are treated using a bisulfite solution (hydrogen sulfite, disulfite). The treatment is such that all non methylated cytosines within the sample are converted to thiamidine, conversely 5-methylated cytosines within the sample remain unmodified.

The treated nucleic acids were then amplified using multiplex PCRs, amplifying 8 fragments per reaction with Cy5 fluorescently labelled primers. PCR primers used are described in Table 2. PCR conditions were as follows.

Reaction solution:

10 ng bisulfite treated DNA

3,5 mM $MgCl_2$

400 μM dNTPs

2 pmol each primer

1 U Hot Star Taq (Qiagen)

Forty cycles were carried out as follows. Denaturation at 95°C for 15 min, followed by annealing at 55°C for 45 sec., primer elongation at 65°C for 2 min. A final elongation at 65°C was carried out for 10 min.

All PCR products from each individual sample were then hybridised to glass slides carrying a pair of immobilised oligonucleotides for each CpG position under analysis. Each of these detection oligonucleotides was designed to hybridise to the bisulphite converted sequence around one CpG site which was either originally unmethylated (TG) or methylated (CG). See Table 3 for further details of all hybridisation oligonucleotides used. Hybridisation conditions were selected to allow the detection of the single nucleotide differences between the TG and CG variants.

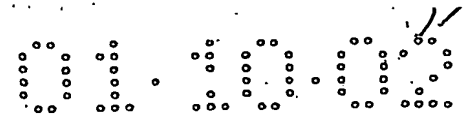
5 μl volume of each multiplex PCR product was diluted in 10 x Ssarc buffer (10 x Ssarc:230 ml 20 x SSC, 180 ml sodium lauroyl sarcosinate solution 20%, dilute to 1000 ml with dH_2O).

The reaction mixture was then hybridised to the detection oligonucleotides as follows. Denaturation at 95°C, cooling down to 10°C, hybridisation at 42°C overnight followed by washing with 10 x Ssarc and dH_2O at 42°C.

Fluorescent signals from each hybridised oligonucleotide were detected using genepix scanner and software. Ratios for the two signals (from the CG oligonucleotide and the TG oligonucleotide used to analyse each CpG position) were calculated based on comparison of intensity of the fluorescent signals.

Example 3

The data obtained according to Example 2 is then sorted into a ranked matrix (as shown in Figure 1) according to CpG methylation differences between the two classes of tissues, using an algorithm. The most significant CpG positions are at the bottom of the matrix with signifi-



cance decreasing towards the top. Black indicates total methylation at a given CpG position, white represents no methylation at the particular position, with degrees of methylation represented in grey, from light (low proportion of methylation) to dark (high proportion of methylation). Each row represents one specific CpG position within a gene and each column shows the methylation profile for the different CpGs for one sample. On the left side a CpG and gene identifier is shown this may be cross referenced with the accompanying table (Table 3) in order to ascertain the gene in question and the detection oligomer used. On the right side p values for the individual CpG positions are shown. The p values are the probabilities that the observed distribution occurred by chance in the data set.

For selected distinctions, the inventors trained a learning algorithm (support vector machine, SVM). The SVM (as discussed by F. Model, P. Adorjan, A. Olek, C. Piepenbrock, Feature selection for DNA methylation based cancer classification. Bioinformatics. 2001 Jun;17 Suppl 1:S157-64) constructs an optimal discriminant between two classes of given training samples. In this case each sample is described by the methylation patterns (CG/TG ratios) at the investigated CpG sites. The SVM was trained on a subset of samples of each class, which were presented with the diagnosis attached. Independent test samples, which were not shown to the SVM before were then presented to evaluate, if the diagnosis can be predicted correctly based on the predictor created in the training round. This procedure was repeated several times using different partitions of the samples, a method called crossvalidation. Please note that all rounds are performed without using any knowledge obtained in the previous runs. The number of correct classifications was averaged over all runs, which gives a good estimate of our test accuracy (percent of correct classified samples over all rounds).

Example 4

The additional data obtained according to Examples 2 and 3 is then sorted into a ranked matrix (as shown in Figures 3 to 10) according to CpG methylation differences between the two classes of tissues, using an algorithm. Figures 3, 5, 7 and 9 are shown in greyscale, wherein the most significant CpG positions are at the bottom of the matrix with significance decreasing towards the top. Black indicates total methylation at a given CpG position, white represents no methylation at the particular position, with degrees of methylation represented in grey, from light (low proportion of methylation) to dark (high proportion of methylation). Each row represents one specific CpG position within a gene and each column shows the methylation profile for the different CpGs for one sample. On the left side a CpG and gene

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identifier is shown this may be cross referenced with the accompanying table (Table 3) in order to ascertain the gene in question and the detection oligomer used. On the right side p values for the individual CpG positions are shown. The p values are the probabilities that the observed distribution occurred by chance in the data set. Figures 4,6,8 and 10 are the red-green versions of the preceding figures (i.e. figures 3,5,7 and 9 respectively). dark grey indicates total methylation at a given CpG position, light grey represents no methylation at the particular position.

For selected distinctions, the inventors trained a learning algorithm (support vector machine, SVM). The SVM (as discussed by F. Model, P. Adorjan, A. Olek, C. Piepenbrock, Feature selection for DNA methylation based cancer classification. Bioinformatics. 2001 Jun;17 Suppl 1:S157-64) constructs an optimal discriminant between two classes of given training samples. In this case each sample is described by the methylation patterns (CG/TG ratios) at the investigated CpG sites. The SVM was trained on a subset of samples of each class, which were presented with the diagnosis attached. Independent test samples, which were not shown to the SVM before were then presented to evaluate, if the diagnosis can be predicted correctly based on the predictor created in the training round. This procedure was repeated several times using different partitions of the samples, a method called crossvalidation. Please note that all rounds are performed without using any knowledge obtained in the previous runs. The number of correct classifications was averaged over all runs, which gives a good estimate of our test accuracy (percent of correct classified samples over all rounds).

Adjuvant setting

Analysis of the methylation patterns of patient samples treated with Tamoxifen as an adjuvant therapy immediately following surgery (see Figure 1) is shown in the matrices according to Figures 3 to 6. In this analysis it can be seen that the genes PTGS2, MSMB, TP53 and CYP2D6 were significantly differentially methylated between the two classes of tissues (responders to therapy and non responders to therapy).

In the classification shown in Figures 3 and 4 the genes TP53 and MSMB were significantly more methylated in non-responders to the drug Tamoxifen, wherein subjects with a disease free survival of less than 36 months were classified as non responders and subjects with a disease free survival of greater than 60 months were classified as responders. This classification was carried out with a sensitivity of 0.84 and a sensitivity of 0.16.

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In the classification shown in Figures 5 and 6 the gene PTGS2 was significantly more methylated in non responders to the drug Tamoxifen wherein subjects with a disease free survival of less than 24 months were classified as non responders and subjects with a disease free survival of greater than 100 months were classified as responders. This classification was carried out with a sensitivity of 0.89 and a sensitivity of 0.48.

Metastatic setting

Analysis of the methylation patterns of patient samples treated with Tamoxifen in a metastatic setting (see Figure 2) is shown in the matrices according to Figures 7 to 10. The subjects analysed in this classification had relapsed following an initial treatment, the subsequent metastasis being treated by Tamoxifen.

In the classification shown in Figures 7 and 8 the genes FGFR1 and PSA were significantly less methylated in non responders to the drug Tamoxifen. Subjects with a progressive disease were classified as non responders and subjects who achieved partial or complete remission were classified as responders. This classification was carried out with a sensitivity of 0.45 and a sensitivity of 0.81.

The sensitivity of this classification could be improved by only analysing those patients who had not undergone chemotherapy. In this classification, shown in Figures 9 and 10 the genes FGFR1, PSA and CGA were significantly less methylated in non responders to the drug Tamoxifen. The sensitivity of the classification was thereby improved to 0.54 and the specificity to 0.85.

Table 2: PCR primers and amplicates

No:	Gene:	Primer:	Amplicate Length:
1	BRCA2 (SEQ ID NO: 1)	ACCCACCCAAACCTAACT (SEQ ID NO: 334) GGTTGGTAGAGATAAAAGGGTA (SEQ ID NO: 333)	388
2	CCND1 (SEQ ID NO: 2)	GATTATAGGGGAGTTTTGTTGA (SEQ ID NO: 335) CACCTCCAACATCCAAATA (SEQ ID NO: 336)	404
3	EDNRB	TCAAAACATCCTCTATCTCTCC	484

No:	Gene:	Primer:	Amplificate Length:
	(SEQ ID NO: 3)	(SEQ ID NO: 338) ATAATTGGGGGTTGTATGTATT (SEQ ID NO: 337)	
4	EGFR (SEQ ID NO: 4)	GGGTTTGGTTGTAATATGGATT (SEQ ID NO: 339) CCCAACACTACCCCTCTAA (SEQ ID NO: 340)	732
5	ERBB2 (SEQ ID NO: 5)	GAGGTAGAGGTTGTGGTGAGT (SEQ ID NO: 341) TCCCAACTTCACTTTCTCC (SEQ ID NO: 342)	528
6	FOS (SEQ ID NO: 6)	ATCCTCCACTTTCTACTTCCA (SEQ ID NO: 344) TTTtagggTTATAGGGAAAGGT (SEQ ID NO: 343)	500
7	NFKB1 (SEQ ID NO: 7)	TTGTAGAATGAAAAGTAGAGTGTG (SEQ ID NO: 345) ACCTTAAAAACCCCAACAAT (SEQ ID NO: 346)	485
8	X51730 PGR (SEQ ID NO: 8)	TTTGGGAATGGGTTGTAT (SEQ ID NO: 347) CTACCCTTAACCTCCATCCTA (SEQ ID NO: 348)	369
9	TP53 (SEQ ID NO: 9)	GAGTAGGTAGTTGTTGGGTTTC (SEQ ID NO: 349) ACCCCTAATTAACTTCTCA (SEQ ID NO: 350)	702
10	TP73 (SEQ ID NO: 10)	AGTAAATAGTGGGTGAGTTATGAA (SEQ ID NO: 351) GAAAAACCTCTAAAACTACTCTCC (SEQ ID NO: 352)	607
11	ESR1 (SEQ ID NO: 11)	AGGGGGAATTAAATAGAAAGAG (SEQ ID NO: 353) CAATAAAACCATCCCAAATACT (SEQ ID NO: 354)	662
12	SERPINE1 (SEQ ID NO: 12)	GGGGTATAGAGAGAGTTTGGAT (SEQ ID NO: 355) ACAATTAAACAAACCCCAATAA (SEQ ID NO: 356)	492
13	CALM1 (SEQ ID NO: 13)	AAAAACTCTAACCCTTCTCAAA (SEQ ID NO: 358) TATTTTtagTTTGGGGTGTTGT (SEQ ID NO: 357)	414
14	CSNK2B (SEQ ID NO: 14)	TACCCCTCACCATTACTCTAAC (SEQ ID NO: 360) TAGTTTTGTGTTTATTGGGTGA (SEQ ID NO: 359)	437
15	FGFR1 (SEQ ID NO: 15)	AGGGAGTTAGTGGTGTGGTAT (SEQ ID NO: 361)	367

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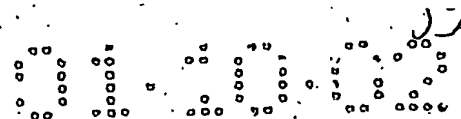
No:	Gene:	Primer:	Amplificate Length:
		CCTTTACCCTTCTCAAATCTAA (SEQ ID NO: 362)	
16	MKI67 (SEQ ID NO: 16)	CCAATACTCTACAACCATCAAA (SEQ ID NO: 364) GGGAAGTTGAAGTAGGAAGAT (SEQ ID NO: 363)	499
17	NPM1 (SEQ ID NO: 17)	AAGGAAGGAGGAAGTAATTTGT (SEQ ID NO: 365) TTACACCAACCCCTAAACTAAC (SEQ ID NO: 366)	454
18	MAPK1 (SEQ ID NO: 18)	TTTAGATAATTTTAGGATGGGG (SEQ ID NO: 367) TTCTCATTCACAAAAACAAAA (SEQ ID NO: 368)	743
19	SYK (SEQ ID NO: 19)	GTGGGTTTTGGGTAGTTATAGA (SEQ ID NO: 369) TAACCTCCTCTCCTTACCAA (SEQ ID NO: 370)	485
20	TK2 (SEQ ID NO: 20)	ATACAACCTCAAATCCTATCCA (SEQ ID NO: 372) AGGGAGAAGGAAGTTATTTGTT (SEQ ID NO: 371)	485
21	HSPB1 (SEQ ID NO: 21)	CCTACCTCTAACCCTTCTCAAT (SEQ ID NO: 374) AAGAGGGTTTAGTTTTTATTG (SEQ ID NO: 373)	216
22	TES (SEQ ID NO: 22)	AGGTTGGGGATTTTAGTTTTT (SEQ ID NO: 375) ACCTTCTTCACTTTATTTTCCA (SEQ ID NO: 376)	448
23	SDC4 (SEQ ID NO: 23)	CCTAACTACCCTCATTCTTT (SEQ ID NO: 378) AGTTGGGGAAATTAAGGTTTAG (SEQ ID NO: 377)	269
24	PITX2 (SEQ ID NO: 24)	TCCTCAACTCTACAAACCTAAAA (SEQ ID NO: 380) GTAGGGGAGGGAAGTAGATGT (SEQ ID NO: 379)	408
25	GPR37 (SEQ ID NO: 25)	ACTTATTTTCTTTCTCTAAAAAC (SEQ ID NO: 382) TATGGTTTGGTGAGGGTATATT (SEQ ID NO: 381)	489
26	FGF1 (SEQ ID NO: 26)	AGTTGTGTTTAATTGGGAAGAG (SEQ ID NO: 383) CTTATCCCATCCACTATACCAT (SEQ ID NO: 384)	420
27	GRIN2D (SEQ ID NO: 27)	ATAGTTTGTGGTTTGGATTTT (SEQ ID NO: 385) AAAACCTTCCCTAACTTCAAT	435

No:	Gene:	Primer:	Amplificate Length:
		(SEQ ID NO: 386)	
28	CTSB (SEQ ID NO: 28)	AAAATTCCATCAAATAACCATAA (SEQ ID NO: 388) AAAAAAGGAAGGTAGTAGGATTGT (SEQ ID NO: 387)	450
29	CTSD (SEQ ID NO: 29)	ATACAACCCTCCAACCTTCTAC (SEQ ID NO: 390) AAGGGGTTTTTAAGGAAATG (SEQ ID NO: 389)	498
30	PLAUR (SEQ ID NO: 30)	TGGTTAAAATGGAGGGTTTAAT (SEQ ID NO: 391) CCCCAAATTACCTAAATACAAA (SEQ ID NO: 392)	348
31	PSA (SEQ ID NO: 31)	TAAGAGAGAGGAGTTGAGGTTT (SEQ ID NO: 393) CCAAAATTAACCTACCTACCTAA (SEQ ID NO: 394)	478
32	CGA (SEQ ID NO: 32)	TAGTGGTATAAGTTTGGAAATGTT (SEQ ID NO: 395) TCCACCTACATCTAAACCCTAA (SEQ ID NO: 396)	364
33	CYP2D6 (SEQ ID NO: 33)	TAAGGGTTTGGAGTAGGAAGTA (SEQ ID NO: 397) CACATACAACAAAATTACCCAA (SEQ ID NO: 398)	403
34	CYP3A4 (SEQ ID NO: 34)	TATCACCACCTTCCCATATTTA (SEQ ID NO: 400) GTTTGATGAATGGATTGTATGA (SEQ ID NO: 399)	484
35	TK1 (SEQ ID NO: 35)	ACCTCTACAAACATCTTATTCCA (SEQ ID NO: 402) TTGGGGGAGTTAGGTAGTATAG (SEQ ID NO: 401)	487
36	RENB (SEQ ID NO: 36)	TTTGGTAGGGTTAAGGTTTTTA (SEQ ID NO: 403) CTTACTCATCCCTCCTACTCC (SEQ ID NO: 404)	350
37	F12 (SEQ ID NO: 37)	TAGGTTTAGGAGGGTAGTTTGA (SEQ ID NO: 405) CTCTCACAACCCAAAAATACA (SEQ ID NO: 406)	450
38	REN (SEQ ID NO: 38)	ACCTACTCCAAAAATCACAAAA (SEQ ID NO: 408) TATGTGGAAAAGTTAGGGTGT (SEQ ID NO: 407)	489
39	EBAG9 (SEQ ID NO: 39)	CCAAACTCATTAACCTCCA (SEQ ID NO: 410) AATGTTTTAGAGGTTAGGGTTG (SEQ ID NO: 409)	463

No:	Gene:	Primer:	Amplificate Length:
40	MSMB (SEQ ID NO: 40)	GTTTGTAGGATGGTTTGATTT (SEQ ID NO: 411) TATATTTACCTTATCCCCACCC (SEQ ID NO: 412)	324
41	X15323 angiotensino gen gene 5'region and exon 1 (SEQ ID NO: 41)	AAACTCTCCCCTACCCTCTAC (SEQ ID NO: 414) GATGGAGTTGTTTTTAGGTTGT (SEQ ID NO: 413)	374
42	ZNF147 (SEQ ID NO: 42)	TTTGTGTAAATAAGATGTGGGA (SEQ ID NO: 415) TAAACCCCTACAAAACCTACCAA (SEQ ID NO: 416)	484
43	EBBP (SEQ ID NO: 43)	GTATTTGTTTTTGGTGAGGGT (SEQ ID NO: 417) ATCATCTTCCTAAACATTCCAA (SEQ ID NO: 418)	482
44	CALR (SEQ ID NO: 44)	TAAATCACAACCATTAAACCAAA (SEQ ID NO: 420) ATAAGAGGGGAGGAAGGTTTA (SEQ ID NO: 419)	490
45	BCAR1 (SEQ ID NO: 45)	AATTCTTCCTTCTATCTCCCTC (SEQ ID NO: 422) TTTATTTTTTGGGAAGGTTGTT (SEQ ID NO: 421)	499
46	COX7A2L (SEQ ID NO: 46)	AATCCTAAAAACCCTAACTTTTAAT (SEQ ID NO: 424) GGAGGTGTAAGGAGAATAGAGA (SEQ ID NO: 423)	398
47	AF174646 glandular kallikrein gene, promoter re- gion and partial se- quence (SEQ ID NO: 47)	CCAATTCATCATTCAACATCTA (SEQ ID NO: 426) ATTTATTTGGGAGGATAGTGG (SEQ ID NO: 425)	325
48	KLK3 (SEQ ID NO: 48)	TTGGAGTGTAAGGATTTAGGT (SEQ ID NO: 427) AACCCACATAATAACACAACTCT (SEQ ID NO: 428)	387
49	AKR1B1 (SEQ ID NO: 49)	CAACAAAAACATTCTTCTAAACTC (SEQ ID NO: 430) AGGTATTTAATTTTAGGATGGGT (SEQ ID NO: 429)	446
50	TGM4 (SEQ ID NO: 50)	AATCCTAACTTTTAATCACCCA (SEQ ID NO: 432) GAGAGGGTAATGGTTTTAGGTA (SEQ ID NO: 431)	435
51	AR (SEQ ID NO: 51)	AATATAGGGAGGTTTAGGGTTT (SEQ ID NO: 433)	424

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No:	Gene:	Primer:	Amplificate Length:
		TAACCATACATTCTCATCCAA (SEQ ID NO: 434)	
52	HSPA1A (SEQ ID NO: 52)	AACCTTTCAAATTCACAATCA (SEQ ID NO: 436) GGATTTATTGGAGGGGATAG (SEQ ID NO: 435)	495
53	FKBP4 (SEQ ID NO: 53)	TTTTTAAGTAGGGAAGGGTTT (SEQ ID NO: 437) TCCTTCTAACTACCTACCCCC (SEQ ID NO: 438)	308
54	ESR2 (SEQ ID NO: 54)	AAACCTTCCCAATAACCTCTTA (SEQ ID NO: 440) TAGAGGGGAGTAGTGTGTTGAGT (SEQ ID NO: 439)	471
55	IGF1 (SEQ ID NO: 55)	TACCCTTCTCCCAAATAATAA (SEQ ID NO: 442) GTATTAAAGGAATATGGGGGAT (SEQ ID NO: 441)	402
56	VTN (SEQ ID NO: 56)	GTTATTTGGGTAAATGTAGGGA (SEQ ID NO: 443) TCTATCCCCTCAAACCTAAAAA (SEQ ID NO: 444)	492
57	CTSL (SEQ ID NO: 57)	CTACACCCACCCTTAAATAAAA (SEQ ID NO: 446) ITAGTGGAATTTGGAGGAAGTAG (SEQ ID NO: 445)	328
58	TGFB3 (SEQ ID NO: 58)	CCTACTAAAAATCAAAACCCAA (SEQ ID NO: 448) AAGGTGGTGTAAGTGGATAGAG (SEQ ID NO: 447)	369
59	MAPKAPK5 (SEQ ID NO: 59)	AAACCTACCTCCCCAACTAA (SEQ ID NO: 450) ATTTTTGGTTTTAGGGTTGTAA (SEQ ID NO: 449)	495
60	PCAF (SEQ ID NO: 60)	GGATAAATGATTGAGAGGTTGT (SEQ ID NO: 451) CCTCCCTTAATTCTCCTACC (SEQ ID NO: 452)	369
61	NCOA3 (SEQ ID NO: 61)	AAGGGGGTGTGTTGTAGATT (SEQ ID NO: 453) CCTAACCTACCCTTAATTTTT (SEQ ID NO: 454)	330
62	PRKCD (SEQ ID NO: 62)	CTTAACCCATCCCAATCA (SEQ ID NO: 456) GATAGAAGGATTTTAGTTTTTATTGTT (SEQ ID NO: 455)	322
63	PTGS2 (SEQ ID NO: 63)	GATTTTTGGAGAGGAAGTTAAG (SEQ ID NO: 458) AAAACATAAAACCAAACCCATA	381



No:	Gene:	Primer:	Amplificate Length:
		(SEQ ID NO: 457)	

Table 3: Hybridisation oligonucleotides.

No:	Gene	Oligo:
1	BRCA2 (SEQ ID NO: 1)	TATAAGTTCGCGTTGG (SEQ ID NO: 459)
2	BRCA2 (SEQ ID NO: 1)	TTATAAGTTTGTGTTGGT (SEQ ID NO: 460)
3	BRCA2 (SEQ ID NO: 1)	TATTCGAGGCGTAGTA (SEQ ID NO: 461)
4	BRCA2 (SEQ ID NO: 1)	TATTTGAGGTGTAGTAGA (SEQ ID NO: 462)
5	BRCA2 (SEQ ID NO: 1)	TTATCGTCGTAAAAGAT (SEQ ID NO: 463)
6	BRCA2 (SEQ ID NO: 1)	GATTTATTGTTGTAAAAGAT (SEQ ID NO: 464)
7	BRCA2 (SEQ ID NO: 1)	ATTCGTTTTAGAGGCGTA (SEQ ID NO: 465)
8	BRCA2 (SEQ ID NO: 1)	ATTTGTTTTAGAGGTGTA (SEQ ID NO: 466)
9	BRCA2 (SEQ ID NO: 1)	TAAGAAGTCGCGGTAG (SEQ ID NO: 467)
10	BRCA2 (SEQ ID NO: 1)	GTAAGAAGTTGTGGTAG (SEQ ID NO: 468)
11	CCND1 (SEQ ID NO: 2)	TGTCGGCGTAGTAGTA (SEQ ID NO: 469)
12	CCND1 (SEQ ID NO: 2)	TGTTGGTGTAGTAGTAG (SEQ ID NO: 470)
13	CCND1 (SEQ ID NO: 2)	AGAAGCGAGAGTCGAG (SEQ ID NO: 471)
14	CCND1 (SEQ ID NO: 2)	TAGAAGTGAGAGTTGAG (SEQ ID NO: 472)
15	CCND1 (SEQ ID NO: 2)	TTTTTAACGATCGGGT (SEQ ID NO: 473)
16	CCND1 (SEQ ID NO: 2)	TTAATGATTGGGTGTTG (SEQ ID NO: 474)
17	CCND1 (SEQ ID NO: 2)	TGTCGTTTATGCGGAA (SEQ ID NO: 475)
18	CCND1 (SEQ ID NO: 2)	TGTTGTTTATGTGGAAG (SEQ ID NO: 476)
19	CCND1 (SEQ ID NO: 2)	GGAAGATCGTCGTTAT (SEQ ID NO: 477)
20	CCND1 (SEQ ID NO: 2)	AGATTGTTGTTATTTGGA (SEQ ID NO: 478)
21	EDNRB (SEQ ID NO: 3)	GTTTATTCGAGAAATGT (SEQ ID NO: 479)

No:	Gene	Oligo:
22	EDNRB (SEQ ID NO: 3)	TGTTTTATTTGAGAAATGT (SEQ ID NO: 480)
23	EDNRB (SEQ ID NO: 3)	GGAATGTTCTGTGTTTA (SEQ ID NO: 481)
24	EDNRB (SEQ ID NO: 3)	GGAATGTTTGTGTTTAT (SEQ ID NO: 482)
25	EDNRB (SEQ ID NO: 3)	TAGGGGTCGATTTTAA (SEQ ID NO: 483)
26	EDNRB (SEQ ID NO: 3)	TTAGGGGTTGATTTTAA (SEQ ID NO: 484)
27	EDNRB (SEQ ID NO: 3)	AATATAATCGGTTAGGTTA (SEQ ID NO: 485)
28	EDNRB (SEQ ID NO: 3)	GATAATATAATTGGTTAGGT (SEQ ID NO: 486)
29	EGFR (SEQ ID NO: 4)	AAGTTTTTCGCGAGTTT (SEQ ID NO: 487)
30	EGFR (SEQ ID NO: 4)	AAAGTTTTTGTGAGTTT (SEQ ID NO: 488)
31	EGFR (SEQ ID NO: 4)	GTATTATTTCTGGACGTT (SEQ ID NO: 489)
32	EGFR (SEQ ID NO: 4)	TATTTTGGATGTTTGGA (SEQ ID NO: 490)
33	EGFR (SEQ ID NO: 4)	TTTCGAGAGGGTTTCGTA (SEQ ID NO: 491)
34	EGFR (SEQ ID NO: 4)	TTTTGAGAGGGTTTGTGA (SEQ ID NO: 492)
35	EGFR (SEQ ID NO: 4)	ATTATTCGACGTTGGT (SEQ ID NO: 493)
36	EGFR (SEQ ID NO: 4)	TTATTATTTGATGTTGGTT (SEQ ID NO: 494)
37	EGFR (SEQ ID NO: 4)	GTAAGTTCGCGGGGAT (SEQ ID NO: 495)
38	EGFR (SEQ ID NO: 4)	GTAAGTTTGTGGGGAT (SEQ ID NO: 496)
39	ERBB2 (SEQ ID NO: 5)	AGATCGCGTTATTGTT (SEQ ID NO: 497)
40	ERBB2 (SEQ ID NO: 5)	AGAGATTGTGTTATTGTT (SEQ ID NO: 498)
41	ERBB2 (SEQ ID NO: 5)	ATTTCGGATTTCTGGGGGA (SEQ ID NO: 499)
42	ERBB2 (SEQ ID NO: 5)	AATTTATTTTGGATTTTGGG (SEQ ID NO: 500)
43	ERBB2 (SEQ ID NO: 5)	GGATTTTTCGAGGAAAA (SEQ ID NO: 501)
44	ERBB2 (SEQ ID NO: 5)	GGGATTTTTCGAGGAAA (SEQ ID NO: 502)
45	ERBB2 (SEQ ID NO: 5)	TAGGTTTTCGCGAAGA (SEQ ID NO: 503)
46	ERBB2 (SEQ ID NO: 5)	TTTGTGTGAAGAGAGG (SEQ ID NO: 504)

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No:	Gene	Oligo:
47	FOS (SEQ ID NO: 6)	TTTCGGGTTTCGAGTAA (SEQ ID NO: 505)
48	FOS (SEQ ID NO: 6)	TTTTGGGTTTGAGTAATA (SEQ ID NO: 506)
49	FOS (SEQ ID NO: 6)	TTTCGAAGGAATGCGTT (SEQ ID NO: 507)
50	FOS (SEQ ID NO: 6)	TTTTGAAGGAATGTGTT (SEQ ID NO: 508)
51	FOS (SEQ ID NO: 6)	AGAAACGGTATCGAGT (SEQ ID NO: 509)
52	FOS (SEQ ID NO: 6)	GGAGAAATGGTATTGAG (SEQ ID NO: 510)
53	FOS (SEQ ID NO: 6)	AATTGTTTCGCGGGTTGTA (SEQ ID NO: 511)
54	FOS (SEQ ID NO: 6)	AATTGTTTGTGGGTTGTA (SEQ ID NO: 512)
55	NFKB1 (SEQ ID NO: 7)	TATAGGCGTTCGTTATT (SEQ ID NO: 513)
56	NFKB1 (SEQ ID NO: 7)	GGATTATAGGTGTTTGTT (SEQ ID NO: 514)
57	NFKB1 (SEQ ID NO: 7)	TATTACGTTTCGGTTAATT (SEQ ID NO: 515)
58	NFKB1 (SEQ ID NO: 7)	ATTATGTTTGGTTAATTTT (SEQ ID NO: 516)
59	NFKB1 (SEQ ID NO: 7)	AGTTATCGTATTCGGTT (SEQ ID NO: 517)
60	NFKB1 (SEQ ID NO: 7)	TGAGTTATTGTATTTGGT (SEQ ID NO: 518)
61	NFKB1 (SEQ ID NO: 7)	TTTAGTTTTTCGGAGTTA (SEQ ID NO: 519)
62	NFKB1 (SEQ ID NO: 7)	TTAGTTTTTTGGAGTTAAA (SEQ ID NO: 520)
63	NFKB1 (SEQ ID NO: 7)	AAAGGTCGATTGTTGG (SEQ ID NO: 521)
64	NFKB1 (SEQ ID NO: 7)	TAAAGGTTGATTGTTGG (SEQ ID NO: 522)
65	X51730 PGR (SEQ ID NO: 8)	TATCGAGAGGTTTCGAT (SEQ ID NO: 523)
66	X51730 PGR (SEQ ID NO: 8)	TGTATTGAGAGGTTTGAT (SEQ ID NO: 524)
67	X51730 PGR (SEQ ID NO: 8)	GAGTCGCGTGTTATTA (SEQ ID NO: 525)
68	X51730 PGR (SEQ ID NO: 8)	AAAGGAGTTGTGTGTT (SEQ ID NO: 526)
69	X51730 PGR (SEQ ID NO: 8)	AAATTGTCGTCGTAGT (SEQ ID NO: 527)
70	X51730 PGR (SEQ ID NO: 8)	ATTAAATTGTTGTTGTAGT (SEQ ID NO: 528)
71	X51730 PGR (SEQ ID NO: 8)	TGTCGTTTCGTTGGGATAA (SEQ ID NO: 529)

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No:	Gene	Oligo:
72	X51730 PGR (SEQ ID NO: 8)	TGTTGTTTGTGTTGGGATAA (SEQ ID NO: 530)
73	TP53 (SEQ ID NO: 9)	TTTTTACGACGGTGAT (SEQ ID NO: 961)
74	TP53 (SEQ ID NO: 9)	TGTTTTTTATGATGGTGA (SEQ ID NO: 962)
75	TP53 (SEQ ID NO: 9)	GAAAATCGTTGGGGTT (SEQ ID NO: 531)
76	TP53 (SEQ ID NO: 9)	GGAAAATTGTTGGGGT (SEQ ID NO: 532)
77	TP53 (SEQ ID NO: 9)	AGTCGGTTTAAAGCGTA (SEQ ID NO: 533)
78	TP53 (SEQ ID NO: 9)	AGTTGGTTTAAAGTGTAT (SEQ ID NO: 534)
79	TP53 (SEQ ID NO: 9)	AGTTTTCGGTTTCGTTAGA (SEQ ID NO: 535)
80	TP53 (SEQ ID NO: 9)	AGTTTTTGTTTTGTTAGA (SEQ ID NO: 536)
81	TP73 (SEQ ID NO: 10)	GTGCGAGTTAGTCGGA (SEQ ID NO: 537)
82	TP73 (SEQ ID NO: 10)	GTGTGAGTTAGTTGGA (SEQ ID NO: 538)
83	TP73 (SEQ ID NO: 10)	TATCGGTTTCGGAGTTA (SEQ ID NO: 539)
84	TP73 (SEQ ID NO: 10)	AGGATATTGGTTTGGAG (SEQ ID NO: 540)
85	TP73 (SEQ ID NO: 10)	AGAGTCGTTTCGGAATT (SEQ ID NO: 541)
86	TP73 (SEQ ID NO: 10)	TGAGAGTTGTTTGAAT (SEQ ID NO: 542)
87	ESR1 (SEQ ID NO: 11)	TTTTCGCGTTTATTTTAA (SEQ ID NO: 543)
88	ESR1 (SEQ ID NO: 11)	TTTTTTGTGTTTATTTAAGT (SEQ ID NO: 544)
89	ESR1 (SEQ ID NO: 11)	AGTAGATTTTCGTGCGTT (SEQ ID NO: 545)
90	ESR1 (SEQ ID NO: 11)	AGTAGATTTTGTGTGTT (SEQ ID NO: 546)
91	ESR1 (SEQ ID NO: 11)	AGTAGCGACGATAAGT (SEQ ID NO: 547)
92	ESR1 (SEQ ID NO: 11)	TTTAGTAGTGATGATAAGT (SEQ ID NO: 548)
93	ESR1 (SEQ ID NO: 11)	GATCGTTTTAAATCGAGT (SEQ ID NO: 549)
94	ESR1 (SEQ ID NO: 11)	GGATTGTTTTAAATTGAGT (SEQ ID NO: 550)
95	ESR1 (SEQ ID NO: 11)	TATCGGATTCGTAGGT (SEQ ID NO: 551)
96	ESR1 (SEQ ID NO: 11)	TTTATTGGATTTGTAGGTT (SEQ ID NO: 552)

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No:	Gene	Oligo:
97	SERPINE1 (SEQ ID NO: 12)	AGTCGTGTATTATCGGA (SEQ ID NO: 553)
98	SERPINE1 (SEQ ID NO: 12)	AGTTGTGTATTATTGGAG (SEQ ID NO: 554)
99	SERPINE1 (SEQ ID NO: 12)	AGGCGGTCGGGTATAT (SEQ ID NO: 555)
100	SERPINE1 (SEQ ID NO: 12)	AGGTGGTTGGGTATAT (SEQ ID NO: 556)
101	SERPINE1 (SEQ ID NO: 12)	TAGAGTATCGGGTGA (SEQ ID NO: 557)
102	SERPINE1 (SEQ ID NO: 12)	AGAGTATTGGGTGGAT (SEQ ID NO: 558)
103	SERPINE1 (SEQ ID NO: 12)	AGGATATTCGGGAGAG (SEQ ID NO: 559)
104	SERPINE1 (SEQ ID NO: 12)	AGGATATTTGGGAGAGA (SEQ ID NO: 560)
105	CALM1 (SEQ ID NO: 13)	TGGCGTTCGTTTATTAA (SEQ ID NO: 561)
106	CALM1 (SEQ ID NO: 13)	TGGTGTGTTGTTTATTAAA (SEQ ID NO: 562)
107	CALM1 (SEQ ID NO: 13)	AGTAGTATTCGAGTTAAAT (SEQ ID NO: 563)
108	CALM1 (SEQ ID NO: 13)	AGTAGTATTTGAGTTAAATT (SEQ ID NO: 564)
109	CALM1 (SEQ ID NO: 13)	TAGAGGACGAGGTAGT (SEQ ID NO: 565)
110	CALM1 (SEQ ID NO: 13)	TAGAGGATGAGGTAGTT (SEQ ID NO: 566)
111	CALM1 (SEQ ID NO: 13)	TTAGTTGTCGAGGAGA (SEQ ID NO: 567)
112	CALM1 (SEQ ID NO: 13)	TTTAGTTGTTGAGGAGA (SEQ ID NO: 568)
113	CSNK2B (SEQ ID NO: 14)	TGATTTTCGAATTTTGGT (SEQ ID NO: 569)
114	CSNK2B (SEQ ID NO: 14)	TGATTTTGAATTTTGGT (SEQ ID NO: 570)
115	CSNK2B (SEQ ID NO: 14)	GAACGTTTGTAGTGCCTT (SEQ ID NO: 571)
116	CSNK2B (SEQ ID NO: 14)	GGAATGTTTGTAGTGTGT (SEQ ID NO: 572)
117	CSNK2B (SEQ ID NO: 14)	GTGCGAATTAGTTTCGTT (SEQ ID NO: 573)
118	CSNK2B (SEQ ID NO: 14)	GTGTGAATTAGTTTGT (SEQ ID NO: 574)
119	CSNK2B (SEQ ID NO: 14)	ATTTTCGGTTAATTCGTT (SEQ ID NO: 575)
120	CSNK2B (SEQ ID NO: 14)	ATTTTGGTTAATTTGTTG (SEQ ID NO: 576)
121	FGFR1 (SEQ ID NO: 15)	GTATTTTCGTTGGTTAAGT (SEQ ID NO: 941)

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No:	Gene	Oligo:
122	FGFR1 (SEQ ID NO: 15)	GTGTATTTTGTGTTAA (SEQ ID NO: 942)
123	FGFR1 (SEQ ID NO: 15)	ATGTGAACGAAGTTAAG (SEQ ID NO: 943)
124	FGFR1 (SEQ ID NO: 15)	ATGTGAATGAAGTTAAGA (SEQ ID NO: 944)
125	FGFR1 (SEQ ID NO: 15)	TATGTTTCGGGGAAGT (SEQ ID NO: 577)
126	FGFR1 (SEQ ID NO: 15)	TATGTTTGGGGAAGTA (SEQ ID NO: 578)
127	FGFR1 (SEQ ID NO: 15)	TATGATTCGTTTTTAAGA (SEQ ID NO: 579)
128	FGFR1 (SEQ ID NO: 15)	AGTTATGATTTGTTTTTAA (SEQ ID NO: 580)
129	MKI67 (SEQ ID NO: 16)	TTGAGATCGCGTTATT (SEQ ID NO: 581)
130	MKI67 (SEQ ID NO: 16)	AGTTGAGATTGTGTTATT (SEQ ID NO: 582)
131	MKI67 (SEQ ID NO: 16)	AGGATCGTTTGAGTCGGG (SEQ ID NO: 583)
132	MKI67 (SEQ ID NO: 16)	AGGATTGTTTGAGTTGGG (SEQ ID NO: 584)
133	MKI67 (SEQ ID NO: 16)	AAATTCGTTAGGCGTG (SEQ ID NO: 585)
134	MKI67 (SEQ ID NO: 16)	AATTTGTTAGGTGTGGT (SEQ ID NO: 586)
135	MKI67 (SEQ ID NO: 16)	TGAGTTCGGGAGTTTA (SEQ ID NO: 587)
136	MKI67 (SEQ ID NO: 16)	TTGAGTTTGGGAGTTTA (SEQ ID NO: 588)
137	NPM1 (SEQ ID NO: 17)	AGTAGTATTCGTTTATTTT (SEQ ID NO: 589)
138	NPM1 (SEQ ID NO: 17)	GAGTAGTATTTGTTTATTTT (SEQ ID NO: 590)
139	NPM1 (SEQ ID NO: 17)	ATTTAAGTGC GTGTTGT (SEQ ID NO: 591)
140	NPM1 (SEQ ID NO: 17)	ATTTAAGTGTGTTGT (SEQ ID NO: 592)
141	NPM1 (SEQ ID NO: 17)	GTTTTTCGATGGAAGAT (SEQ ID NO: 593)
142	NPM1 (SEQ ID NO: 17)	TGTTTTTTGATGGAAGAT (SEQ ID NO: 594)
143	NPM1 (SEQ ID NO: 17)	TGATGGACGTGGATAT (SEQ ID NO: 595)
144	NPM1 (SEQ ID NO: 17)	TTGATGGATGTGGATAT (SEQ ID NO: 596)
145	MAPK1 (SEQ ID NO: 18)	AGTTTTACGTTTCGATT (SEQ ID NO: 597)
146	MAPK1 (SEQ ID NO: 18)	TGTAGTTTTATGTTTGT (SEQ ID NO: 598)

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No:	Gene	Oligo:
147	MAPK1 (SEQ ID NO: 18)	AAGAAACGATCGAATTT (SEQ ID NO: 599)
148	MAPK1 (SEQ ID NO: 18)	AAATGATTGAATTTGGGA (SEQ ID NO: 600)
149	MAPK1 (SEQ ID NO: 18)	ATTTATTTTTCGTGTGTAT (SEQ ID NO: 601)
150	MAPK1 (SEQ ID NO: 18)	AATTTATTTTGTGTGTAT (SEQ ID NO: 602)
151	MAPK1 (SEQ ID NO: 18)	GTGTATCGTGTTTTAAT (SEQ ID NO: 603)
152	MAPK1 (SEQ ID NO: 18)	TGTGTATTGTGTTTAAAT (SEQ ID NO: 604)
153	SYK (SEQ ID NO: 19)	GAAGTTATCGCGTTGG (SEQ ID NO: 605)
154	SYK (SEQ ID NO: 19)	AGAAGTTATTGTGTTGG (SEQ ID NO: 606)
155	SYK (SEQ ID NO: 19)	GATCGATGCGGTTTAT (SEQ ID NO: 607)
156	SYK (SEQ ID NO: 19)	GGGATTGATGTGGTTTA (SEQ ID NO: 608)
157	SYK (SEQ ID NO: 19)	GTTCGGCGGGAGGAGA (SEQ ID NO: 609)
158	SYK (SEQ ID NO: 19)	GTTTGGTGGGAGGAGA (SEQ ID NO: 610)
159	SYK (SEQ ID NO: 19)	AGTCGATTTTCGTTTAG (SEQ ID NO: 611)
160	SYK (SEQ ID NO: 19)	TAGTTGATTTTGTTTAGT (SEQ ID NO: 612)
161	SYK (SEQ ID NO: 19)	GGAAGAGTCGCGGGTT (SEQ ID NO: 613)
162	SYK (SEQ ID NO: 19)	GGAAGAGTTGTGGGT (SEQ ID NO: 614)
163	TK2 (SEQ ID NO: 20)	AAGTTTCGAGTGTGAT (SEQ ID NO: 615)
164	TK2 (SEQ ID NO: 20)	AAGTTTGTGAGTGTGATG (SEQ ID NO: 616)
165	TK2 (SEQ ID NO: 20)	TTAAGACGGTGGAGAT (SEQ ID NO: 617)
166	TK2 (SEQ ID NO: 20)	TTTAAAGATGGTGGAGA (SEQ ID NO: 618)
167	TK2 (SEQ ID NO: 20)	GAATTACGGTGATGGT (SEQ ID NO: 619)
168	TK2 (SEQ ID NO: 20)	GAATGAATTATGGTGATG (SEQ ID NO: 620)
169	TK2 (SEQ ID NO: 20)	AGGTAATTGTCGATTAGA (SEQ ID NO: 621)
170	TK2 (SEQ ID NO: 20)	AGGTAATTGTTGATTAGAT (SEQ ID NO: 622)
171	TK2 (SEQ ID NO: 20)	GTGTGTTTTTCGTAAATA (SEQ ID NO: 623)

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No:	Gene	Oligo:
172	TK2 (SEQ ID NO: 20)	GTGTGTTTTTTGTAAATAA (SEQ ID NO: 624)
173	HSPB1 (SEQ ID NO: 21)	GTGTTACGGTAGGGTA (SEQ ID NO: 625)
174	HSPB1 (SEQ ID NO: 21)	GTGTTATGGTAGGGTAT (SEQ ID NO: 626)
175	HSPB1 (SEQ ID NO: 21)	AGTCGTGTTACGGTAG (SEQ ID NO: 627)
176	HSPB1 (SEQ ID NO: 21)	AGTTGTGTTATGGTAGG (SEQ ID NO: 628)
177	HSPB1 (SEQ ID NO: 21)	TTTTTTCGTTAAGGAAAG (SEQ ID NO: 629)
178	HSPB1 (SEQ ID NO: 21)	TTTTTTTGTTAAGGAAAG (SEQ ID NO: 630)
179	TES (SEQ ID NO: 22)	TAGAAGTCGGTTCGTG (SEQ ID NO: 631)
180	TES (SEQ ID NO: 22)	AGAAGTTGGTTTGTGG (SEQ ID NO: 632)
181	TES (SEQ ID NO: 22)	GTTTCGTGGACGTTTAGA (SEQ ID NO: 633)
182	TES (SEQ ID NO: 22)	GTTTGTGGATGTTTAGA (SEQ ID NO: 634)
183	TES (SEQ ID NO: 22)	GATTGGGCGGCGGAAG (SEQ ID NO: 635)
184	TES (SEQ ID NO: 22)	ATTGGGTGGTGGAAGT (SEQ ID NO: 636)
185	TES (SEQ ID NO: 22)	TAGCGGAGTCGGAGGT (SEQ ID NO: 637)
186	TES (SEQ ID NO: 22)	TAGTGGAGTTGGAGGT (SEQ ID NO: 638)
187	TES (SEQ ID NO: 22)	AATTCGGTCGTGGGAT (SEQ ID NO: 639)
188	TES (SEQ ID NO: 22)	AATTGTTGTGGGAT (SEQ ID NO: 640)
189	SDC4 (SEQ ID NO: 23)	TTGCGGGTTATACGAT (SEQ ID NO: 641)
190	SDC4 (SEQ ID NO: 23)	TTTGTGGGTTATATGATT (SEQ ID NO: 642)
191	SDC4 (SEQ ID NO: 23)	GTGAATCGTGTTAAGAT (SEQ ID NO: 643)
192	SDC4 (SEQ ID NO: 23)	TGTGAATTGTGTTAAGAT (SEQ ID NO: 644)
193	PITX2 (SEQ ID NO: 24)	AGTCGGGAGAGCGAAA (SEQ ID NO: 645)
194	PITX2 (SEQ ID NO: 24)	AGTTGGGAGAGTGAAA (SEQ ID NO: 646)
195	PITX2 (SEQ ID NO: 24)	AAGAGTCGGGAGTCGGA (SEQ ID NO: 647)
196	PITX2 (SEQ ID NO: 24)	AAGAGTTGGGAGTTGGA (SEQ ID NO: 648)

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No:	Gene	Oligo:
197	PITX2 (SEQ ID NO: 24)	GGTCGAAGAGTCGGGA (SEQ ID NO: 649)
198	PITX2 (SEQ ID NO: 24)	GGTTGAAGAGTTGGGA (SEQ ID NO: 650)
199	PITX2 (SEQ ID NO: 24)	ATGTTAGCGGGTCGAA (SEQ ID NO: 651)
200	PITX2 (SEQ ID NO: 24)	TAGTGGGTTGAAGAGT (SEQ ID NO: 652)
201	GPR37 (SEQ ID NO: 25)	TTTCGATAGCGTTTGA (SEQ ID NO: 653)
202	GPR37 (SEQ ID NO: 25)	TTTTGATAGTGTGTTGATT (SEQ ID NO: 654)
203	GPR37 (SEQ ID NO: 25)	ATAGCGGAAGATCGGT (SEQ ID NO: 655)
204	GPR37 (SEQ ID NO: 25)	ATAGTGGAAGATTGGTT (SEQ ID NO: 656)
205	GPR37 (SEQ ID NO: 25)	TAGCGGGGAATCGGAG (SEQ ID NO: 657)
206	GPR37 (SEQ ID NO: 25)	TAGTGGGGAATTGGAG (SEQ ID NO: 658)
207	GPR37 (SEQ ID NO: 25)	ATTTATTTTCGTTCCGGTA (SEQ ID NO: 659)
208	GPR37 (SEQ ID NO: 25)	ATTTATTTTGTGTTGGGTA (SEQ ID NO: 660)
209	GPR37 (SEQ ID NO: 25)	ATTCGGTAGTCGTAGT (SEQ ID NO: 661)
210	GPR37 (SEQ ID NO: 25)	ATTTGGTAGTTGTAGTTT (SEQ ID NO: 662)
211	FGF1 (SEQ ID NO: 26)	TATAGGTTTCGCGGATT (SEQ ID NO: 663)
212	FGF1 (SEQ ID NO: 26)	TTATAGGTTTGTGGATTG (SEQ ID NO: 664)
213	FGF1 (SEQ ID NO: 26)	ATTATTCGGGAATTTTGT (SEQ ID NO: 665)
214	FGF1 (SEQ ID NO: 26)	TATTATTTGGAATTTTGT (SEQ ID NO: 666)
215	FGF1 (SEQ ID NO: 26)	AGTTTGTATCGAGAAGT (SEQ ID NO: 667)
216	FGF1 (SEQ ID NO: 26)	TAGTTTGTATTGAGAAGT (SEQ ID NO: 668)
217	FGF1 (SEQ ID NO: 26)	TAGTAACGGGGGTTAT (SEQ ID NO: 669)
218	FGF1 (SEQ ID NO: 26)	TGTAGTAATGGGGGT (SEQ ID NO: 670)
219	FGF1 (SEQ ID NO: 26)	GATTTTTTCGGATGGTA (SEQ ID NO: 671)
220	FGF1 (SEQ ID NO: 26)	ATTTTTTTGGATGGTATAG (SEQ ID NO: 672)
221	GRIN2D (SEQ ID NO: 27)	GAGAGTCGGGATGATT (SEQ ID NO: 673)

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No.	Gene	Oligo:
222	GRIN2D (SEQ ID NO: 27)	GGAGAGTTGGGATGAT (SEQ ID NO: 674)
223	GRIN2D (SEQ ID NO: 27)	AGAGATTTCGATTGGA (SEQ ID NO: 675)
224	GRIN2D (SEQ ID NO: 27)	AAGAGATTTTGATTGGA (SEQ ID NO: 676)
225	GRIN2D (SEQ ID NO: 27)	TAGGGTCGAGATTG (SEQ ID NO: 677)
226	GRIN2D (SEQ ID NO: 27)	TTAGGGTTGAGATTG (SEQ ID NO: 678)
227	GRIN2D (SEQ ID NO: 27)	AGTGTGGCGAATATTG (SEQ ID NO: 679)
228	GRIN2D (SEQ ID NO: 27)	GTGTGGTGAATATTGAA (SEQ ID NO: 680)
229	CTSB (SEQ ID NO: 28)	TGGGATATTCGAGGAAT (SEQ ID NO: 681)
230	CTSB (SEQ ID NO: 28)	GGATATTTGAGGAATTGA (SEQ ID NO: 682)
231	CTSB (SEQ ID NO: 28)	TGATATGCGTTATGGTT (SEQ ID NO: 683)
232	CTSB (SEQ ID NO: 28)	TTGATATGTGTTATGGTT (SEQ ID NO: 684)
233	CTSD (SEQ ID NO: 29)	TAGAGGGCGAGCGGTA (SEQ ID NO: 685)
234	CTSD (SEQ ID NO: 29)	TAGAGGGTGAGTGGTA (SEQ ID NO: 686)
235	CTSD (SEQ ID NO: 29)	GGAGCGCGAAAGTTAT (SEQ ID NO: 687)
236	CTSD (SEQ ID NO: 29)	TAGGGGAGTGTGAAAG (SEQ ID NO: 688)
237	CTSD (SEQ ID NO: 29)	TAGGAGGTCGCGTAGG (SEQ ID NO: 689)
238	CTSD (SEQ ID NO: 29)	TAGGAGGTTGTGTAGG (SEQ ID NO: 690)
239	CTSD (SEQ ID NO: 29)	ATTCGTTTAGATTCTGGG (SEQ ID NO: 691)
240	CTSD (SEQ ID NO: 29)	ATTTGTTTAGATTGTTGGG (SEQ ID NO: 692)
241	PLAUR (SEQ ID NO: 30)	GGTATAGCGGGAAGT (SEQ ID NO: 693)
242	PLAUR (SEQ ID NO: 30)	GGTATAGTGGGAAGTAA (SEQ ID NO: 694)
243	PLAUR (SEQ ID NO: 30)	ATTTATATTTATCGTTAAAGT (SEQ ID NO: 695)
244	PLAUR (SEQ ID NO: 30)	TATATTTATTGTAAAGTTTTT (SEQ ID NO: 696)
245	PLAUR (SEQ ID NO: 30)	AGAGCGATTGTTTCGGG (SEQ ID NO: 697)
246	PLAUR (SEQ ID NO: 30)	AGAGTGATTGTTTGGG (SEQ ID NO: 698)

No:	Gene	Oligo:
247	PLAUR (SEQ ID NO: 30)	GGGTTTTACGTTAGGA (SEQ ID NO: 699)
248	PLAUR (SEQ ID NO: 30)	GGGTTTTATGTTAGGAA (SEQ ID NO: 700)
249	PSA (SEQ ID NO: 31)	TTTCGATTTCGGTTTAGA (SEQ ID NO: 945)
250	PSA (SEQ ID NO: 31)	AATTGTTTTGATTGTT (SEQ ID NO: 946)
251	PSA (SEQ ID NO: 31)	TAATGGGGCGTCGATT (SEQ ID NO: 701)
252	PSA (SEQ ID NO: 31)	TTAATGGGGTGTTGATT (SEQ ID NO: 702)
253	PSA (SEQ ID NO: 31)	TATCGTAGCGGTTAGG (SEQ ID NO: 703)
254	PSA (SEQ ID NO: 31)	TATTGTAGTGGTTAGGAA (SEQ ID NO: 704)
255	PSA (SEQ ID NO: 31)	AGGAACGTTAGTCGTT (SEQ ID NO: 947)
256	PSA (SEQ ID NO: 31)	TAGGAATGTTAGTTGTTT (SEQ ID NO: 948)
257	PSA (SEQ ID NO: 31)	GGTCGTCGTATTATGGA (SEQ ID NO: 949)
258	PSA (SEQ ID NO: 31)	TGGTTGTTGTATTATGGA (SEQ ID NO: 950)
259	CGA (SEQ ID NO: 32)	ATATTTATTTTCGGAAATTT (SEQ ID NO: 705)
260	CGA (SEQ ID NO: 32)	TTATTTTGGAAATTTATAGT (SEQ ID NO: 706)
261	CGA (SEQ ID NO: 32)	TGATTTTGTCGTTATTATT (SEQ ID NO: 707)
262	CGA (SEQ ID NO: 32)	TTGATTTTGTTGTTATTATT (SEQ ID NO: 708)
263	CGA (SEQ ID NO: 32)	TAAATTGACGTTATGGTA (SEQ ID NO: 951)
264	CGA (SEQ ID NO: 32)	AAATTGATGTTATGGTAAA (SEQ ID NO: 952)
265	CGA (SEQ ID NO: 32)	AATTGACGTTATGGTAAT (SEQ ID NO: 953)
266	CGA (SEQ ID NO: 32)	TAAAAATTGATGTTATGGT (SEQ ID NO: 954)
267	CYP2D6 (SEQ ID NO: 33)	AAGTAGCGGTAAGGAT (SEQ ID NO: 963)
268	CYP2D6 (SEQ ID NO: 33)	GAAGTAGTGGTAAGGAT (SEQ ID NO: 964)
269	CYP2D6 (SEQ ID NO: 33)	TAGAGGGCGAAGGTTA (SEQ ID NO: 709)
270	CYP2D6 (SEQ ID NO: 33)	AGAGGGTGAAGGTTAT (SEQ ID NO: 710)
271	CYP2D6 (SEQ ID NO: 33)	TGTATCGGTATTAACGTT (SEQ ID NO: 711)

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No:	Gene	Oligo:
272	CYP2D6 (SEQ ID NO: 33)	TGTATTGGTATTAATGTTG (SEQ ID NO: 712)
273	CYP2D6 (SEQ ID NO: 33)	GTACGTTATTCGTTAGG (SEQ ID NO: 713)
274	CYP2D6 (SEQ ID NO: 33)	TGTATGTTATTTGTTAGGT (SEQ ID NO: 714)
275	CYP3A4 (SEQ ID NO: 34)	AAAAGAGTCGTATAGAGA (SEQ ID NO: 715)
276	CYP3A4 (SEQ ID NO: 34)	AAGAGTTGTATAGAGAGG (SEQ ID NO: 716)
277	CYP3A4 (SEQ ID NO: 34)	GTGTGTCGGGAGTTATA (SEQ ID NO: 717)
278	CYP3A4 (SEQ ID NO: 34)	GTGTGTTGGGAGTTATA (SEQ ID NO: 718)
279	CYP3A4 (SEQ ID NO: 34)	AAATAGGCGATGTTTAA (SEQ ID NO: 719)
280	CYP3A4 (SEQ ID NO: 34)	TAGAAATAGGTGATGTTT (SEQ ID NO: 720)
281	CYP3A4 (SEQ ID NO: 34)	TAAAATTATAGCGTTTTTAA (SEQ ID NO: 721)
282	CYP3A4 (SEQ ID NO: 34)	AAATTATAGTGTTTTTTAAAT (SEQ ID NO: 722)
283	TK1 (SEQ ID NO: 35)	AGGAATTCGGTACGTG (SEQ ID NO: 723)
284	TK1 (SEQ ID NO: 35)	GAGGAATTTGGTATGTG (SEQ ID NO: 724)
285	TK1 (SEQ ID NO: 35)	AATTACGAGTCGGTTT (SEQ ID NO: 725)
286	TK1 (SEQ ID NO: 35)	GGTTAATTATGAGTTGGT (SEQ ID NO: 726)
287	TK1 (SEQ ID NO: 35)	AGGTCGTTTCGTAGTA (SEQ ID NO: 727)
288	TK1 (SEQ ID NO: 35)	TTAAGGTTGTTTTGTAGT (SEQ ID NO: 728)
289	TK1 (SEQ ID NO: 35)	TTTCGGGAAGTTTACGAA (SEQ ID NO: 729)
290	TK1 (SEQ ID NO: 35)	TTTTGGGAAGTTTATGAA (SEQ ID NO: 730)
291	RENBP (SEQ ID NO: 36)	TAGATTTTTCGGTTATTTT (SEQ ID NO: 731)
292	RENBP (SEQ ID NO: 36)	TTTAGATTTTTCGGTTATTT (SEQ ID NO: 732)
293	RENBP (SEQ ID NO: 36)	GGTTTATTCGAGTGGA (SEQ ID NO: 733)
294	RENBP (SEQ ID NO: 36)	TGGTTTATTTGAGTGGA (SEQ ID NO: 734)
295	RENBP (SEQ ID NO: 36)	TAAAGTTTCGTTTTAGGT (SEQ ID NO: 735)
296	RENBP (SEQ ID NO: 36)	TTAAAGTTTGTTTTAGGT (SEQ ID NO: 736)

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No:	Gene	Oligo:
297	RENBP (SEQ ID NO: 36)	GGGATCGCGATTAATT (SEQ ID NO: 737)
298	RENBP (SEQ ID NO: 36)	GGGATTGTGATTAATTTTG (SEQ ID NO: 738)
299	F12 (SEQ ID NO: 37)	TGGATTAACGGACGGA (SEQ ID NO: 739)
300	F12 (SEQ ID NO: 37)	TTGGATTAATGGATGGA (SEQ ID NO: 740)
301	F12 (SEQ ID NO: 37)	GGACGGACGTTATGAG (SEQ ID NO: 741)
302	F12 (SEQ ID NO: 37)	GGATGGATGTTATGAGG (SEQ ID NO: 742)
303	F12 (SEQ ID NO: 37)	ATATTTTCGGTGAGTGT (SEQ ID NO: 743)
304	F12 (SEQ ID NO: 37)	AATATTTTGGTGAGTGT (SEQ ID NO: 744)
305	F12 (SEQ ID NO: 37)	GGGGGGTCGTTATTATA (SEQ ID NO: 745)
306	F12 (SEQ ID NO: 37)	GGGGGGTTGTTATTATA (SEQ ID NO: 746)
307	F12 (SEQ ID NO: 37)	GTTAGTTCGGAAGGTT (SEQ ID NO: 747)
308	F12 (SEQ ID NO: 37)	GGTAGTTTGGAAGGT (SEQ ID NO: 748)
309	REN (SEQ ID NO: 38)	TTATTGCGGGATAGAG (SEQ ID NO: 749)
310	REN (SEQ ID NO: 38)	TTATTGTGGGATAGAGT (SEQ ID NO: 750)
311	REN (SEQ ID NO: 38)	TTTTTAGCGAGGTATTT (SEQ ID NO: 751)
312	REN (SEQ ID NO: 38)	AGTTTTTAGTGAGGTATT (SEQ ID NO: 752)
313	REN (SEQ ID NO: 38)	GTTTGTCGGGAGATTA (SEQ ID NO: 753)
314	REN (SEQ ID NO: 38)	TGTTTGTTGGGAGATTA (SEQ ID NO: 754)
315	REN (SEQ ID NO: 38)	TAATTATCGTTTAAAGGTG (SEQ ID NO: 755)
316	REN (SEQ ID NO: 38)	TTAATTATTGTTTAAAGGTG (SEQ ID NO: 756)
317	EBAG9 (SEQ ID NO: 39)	GTTTGACGTCGAGATT (SEQ ID NO: 757)
318	EBAG9 (SEQ ID NO: 39)	TGATGTTGAGATTGGG (SEQ ID NO: 758)
319	EBAG9 (SEQ ID NO: 39)	TTATCGGGAAATTCGTT (SEQ ID NO: 759)
320	EBAG9 (SEQ ID NO: 39)	TTTATTGGGAAATTTGTT (SEQ ID NO: 760)
321	EBAG9 (SEQ ID NO: 39)	ATTTTATGTTTCGTTTATTT (SEQ ID NO: 761)

No:	Gene	Oligo:
	angiotensinogen gene 5'region and exon 1 (SEQ ID NO: 41)	(SEQ ID NO: 774)
337	ZNF147 (SEQ ID NO: 42)	TAGGTAACGTATAGAGAT (SEQ ID NO: 775)
338	ZNF147 (SEQ ID NO: 42)	TTTAGGTAATGTATAGAGA (SEQ ID NO: 776)
339	ZNF147 (SEQ ID NO: 42)	AGGAGTGTCTGATTTTA (SEQ ID NO: 777)
340	ZNF147 (SEQ ID NO: 42)	AGGAGTGTTGTATTTTAG (SEQ ID NO: 778)
341	ZNF147 (SEQ ID NO: 42)	AGGTTAGCGATTTTGA (SEQ ID NO: 779)
342	ZNF147 (SEQ ID NO: 42)	AGGTTAGTGATTTTGAG (SEQ ID NO: 780)
343	EBBP (SEQ ID NO: 43)	TTTAGGTCGTTTTTATTTAT (SEQ ID NO: 781)
344	EBBP (SEQ ID NO: 43)	TTTAGGTTGTTTTTATTTATA (SEQ ID NO: 782)
345	EBBP (SEQ ID NO: 43)	ATGGTATCGAGTCGTT (SEQ ID NO: 783)
346	EBBP (SEQ ID NO: 43)	TGGTATTGAGTTGTTTAT (SEQ ID NO: 784)
347	EBBP (SEQ ID NO: 43)	GGAATGTTTCGTTAAGT (SEQ ID NO: 785)
348	EBBP (SEQ ID NO: 43)	ATGTTTTGTTAAGTGGG (SEQ ID NO: 786)
349	EBBP (SEQ ID NO: 43)	TTACGAGTGTGACGGA (SEQ ID NO: 787)
350	EBBP (SEQ ID NO: 43)	TTATGAGTGTGATGGAT (SEQ ID NO: 788)
351	CALR (SEQ ID NO: 44)	TGTTTTACGTTGTAGAA (SEQ ID NO: 789)
352	CALR (SEQ ID NO: 44)	GTTTTTATGTTGTAGAAAG (SEQ ID NO: 790)
353	CALR (SEQ ID NO: 44)	AGAGGTACGTGATTGG (SEQ ID NO: 791)
354	CALR (SEQ ID NO: 44)	TAGAGGTATGTGATTGG (SEQ ID NO: 792)
355	CALR (SEQ ID NO: 44)	TTTTTTAATCGGTAAATGA (SEQ ID NO: 793)
356	CALR (SEQ ID NO: 44)	TTTTTTAATTGGTAAATGAA (SEQ ID NO: 794)
357	CALR (SEQ ID NO: 44)	AGATTGTAGTCGTTTTTT (SEQ ID NO: 795)
358	CALR (SEQ ID NO: 44)	AGATTGTAGTTGTTTTTTT (SEQ ID NO: 796)
359	CALR (SEQ ID NO: 44)	AGGTTTAACGATGTGAT (SEQ ID NO: 797)

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No:	Gene	Oligo:
360	CALR (SEQ ID NO: 44)	AGGTTTAATGATGTGATT (SEQ ID NO: 798)
361	BCAR1 (SEQ ID NO: 45)	AGTTTTTCGATTTAGCGTT (SEQ ID NO: 799)
362	BCAR1 (SEQ ID NO: 45)	AGTTTTTGATTTAGTGTTT (SEQ ID NO: 800)
363	BCAR1 (SEQ ID NO: 45)	TGGCGAGTTTTTCGATTTA (SEQ ID NO: 801)
364	BCAR1 (SEQ ID NO: 45)	TGGTGAGTTTTTGATTTA (SEQ ID NO: 802)
365	BCAR1 (SEQ ID NO: 45)	TTTCGATTGTAGACGGT (SEQ ID NO: 803)
366	BCAR1 (SEQ ID NO: 45)	TTTTGATTGTAGATGGTT (SEQ ID NO: 804)
367	BCAR1 (SEQ ID NO: 45)	ATTTATCGCGTGTTTAT (SEQ ID NO: 805)
368	BCAR1 (SEQ ID NO: 45)	GGTATTTATTGTGTGTTTA (SEQ ID NO: 806)
369	COX7A2L (SEQ ID NO: 46)	TTGTTCGAAGATCGTT (SEQ ID NO: 807)
370	COX7A2L (SEQ ID NO: 46)	GTTGTTTGAAGATTGTTT (SEQ ID NO: 808)
371	COX7A2L (SEQ ID NO: 46)	TAGCGTAAGGATTCGGT (SEQ ID NO: 809)
372	COX7A2L (SEQ ID NO: 46)	TTAGTGTAAGGATTGTT (SEQ ID NO: 810)
373	COX7A2L (SEQ ID NO: 46)	AGAGTTCGGTTTTTCGTA (SEQ ID NO: 811)
374	COX7A2L (SEQ ID NO: 46)	AGAGTTTGGTTTTTTGTA (SEQ ID NO: 812)
375	COX7A2L (SEQ ID NO: 46)	ATTCGTATTTGCGGGTTA (SEQ ID NO: 813)
376	COX7A2L (SEQ ID NO: 46)	ATTTGTATTTGTGGGTTA (SEQ ID NO: 814)
377	AF174646 glandular kallikrein gene, promoter re- gion and partial se- quence (SEQ ID NO: 47)	GTTTCGTATAGCGTAGAT (SEQ ID NO: 815)
378	AF174646 glandular kallikrein gene, promoter re- gion and partial se- quence (SEQ ID NO: 47)	GTTTGTATAGTG TAGATGT (SEQ ID NO: 816)
379	AF174646 glandular kallikrein gene, promoter re- gion and partial se-	AGAGGCGCGTTGTAGG (SEQ ID NO: 817)

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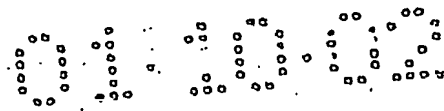
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No:	Gene	Oligo:
	quence (SEQ ID NO: 47)	
380	AF174646 glandular kallikrein gene, promoter re- gion and partial se- quence (SEQ ID NO: 47)	AGAGGTGTGTTGTAGG (SEQ ID NO: 818)
381	AF174646 glandular kallikrein gene, promoter re- gion and partial se- quence (SEQ ID NO: 47)	TTGGGACGTGACGGGA (SEQ ID NO: 819)
382	AF174646 glandular kallikrein gene, promoter re- gion and partial se- quence (SEQ ID NO: 47)	TTGGGATGTGATGGGA (SEQ ID NO: 820)
383	AF174646 glandular kallikrein gene, promoter re- gion and partial se- quence (SEQ ID NO: 47)	TAGGGGACGTACGTTT (SEQ ID NO: 821)
384	AF174646 glandular kallikrein gene, promoter re- gion and partial se- quence (SEQ ID NO: 47)	TATTAGGGGATGTATGTT (SEQ ID NO: 822)
385	KLK3 (SEQ ID NO: 48)	AATCGGGGATCGTATT (SEQ ID NO: 823)
386	KLK3 (SEQ ID NO: 48)	AGAATTGGGGATTGTAT (SEQ ID NO: 824)
387	KLK3 (SEQ ID NO: 48)	ATTTGTATTCGGAGAGT (SEQ ID NO: 825)
388	KLK3 (SEQ ID NO: 48)	TGTATTTGGAGAGTTGT (SEQ ID NO: 826)
389	AKR1B1 (SEQ ID NO: 49)	GTTTCGCGTGGTAGAA (SEQ ID NO: 827)
390	AKR1B1 (SEQ ID NO: 49)	GTTTTGTGTGGTAGAAA (SEQ ID NO: 828)
391	AKR1B1 (SEQ ID NO: 49)	GTTGCGCGAAGGAGTT (SEQ ID NO: 829)
392	AKR1B1 (SEQ ID NO: 49)	GTTGTGTGAAGGAGTT (SEQ ID NO: 830)
393	AKR1B1 (SEQ ID NO: 49)	AGTTCGTGAGGTCGGTA (SEQ ID NO: 831)

No:	Gene	Oligo:
394	AKR1B1 (SEQ ID NO: 49)	AGTTTGTGAGGTTGGTA (SEQ ID NO: 832)
395	AKR1B1 (SEQ ID NO: 49)	ATTTTCGTTTAGTACGGT (SEQ ID NO: 833)
396	AKR1B1 (SEQ ID NO: 49)	GATTTTGTGTTAGTATGGT (SEQ ID NO: 834)
397	TGM4 (SEQ ID NO: 50)	TAATAAAATTTTCGGAATTTT (SEQ ID NO: 835)
398	TGM4 (SEQ ID NO: 50)	TAATAAAATTTTGGGAATTTT (SEQ ID NO: 836)
399	TGM4 (SEQ ID NO: 50)	GGAAGGCGATGTTGAT (SEQ ID NO: 837)
400	TGM4 (SEQ ID NO: 50)	GGAAGGTGATGTTGAT (SEQ ID NO: 838)
401	TGM4 (SEQ ID NO: 50)	ATATAGTCGGTTTTTAGG (SEQ ID NO: 839)
402	TGM4 (SEQ ID NO: 50)	TATAGTTGGTTTTTAGGG (SEQ ID NO: 840)
403	TGM4 (SEQ ID NO: 50)	AGGTATTCGGTAGAGG (SEQ ID NO: 841)
404	TGM4 (SEQ ID NO: 50)	AGGTATTTGGTAGAGGT (SEQ ID NO: 842)
405	AR (SEQ ID NO: 51)	AAGAGGCGAAAAGTAG (SEQ ID NO: 843)
406	AR (SEQ ID NO: 51)	AAGAGGTGAAAAGTAGT (SEQ ID NO: 844)
407	AR (SEQ ID NO: 51)	AGTAAGCGGTTGTATAT (SEQ ID NO: 845)
408	AR (SEQ ID NO: 51)	AAAAGTAAGTGGTTGTAT (SEQ ID NO: 846)
409	AR (SEQ ID NO: 51)	AAATTGAGCGTTTATGT (SEQ ID NO: 847)
410	AR (SEQ ID NO: 51)	ATTGAGTGTTTATGTGTA (SEQ ID NO: 848)
411	HSPA1A (SEQ ID NO: 52)	AGGCGTCGGGAGATTA (SEQ ID NO: 849)
412	HSPA1A (SEQ ID NO: 52)	AGGTGTTGGGAGATTA (SEQ ID NO: 850)
413	HSPA1A (SEQ ID NO: 52)	TTGCGTAATTTGGACGTT (SEQ ID NO: 851)
414	HSPA1A (SEQ ID NO: 52)	TTGTGTAATTTGGATGTT (SEQ ID NO: 852)
415	HSPA1A (SEQ ID NO: 52)	GTTTCGGTTACGTTATAAT (SEQ ID NO: 853)
416	HSPA1A (SEQ ID NO: 52)	GTTTGGTTATGTTATAATGG (SEQ ID NO: 854)
417	HSPA1A (SEQ ID NO: 52)	TTGATATCGTTCGGTTA (SEQ ID NO: 855)
418	HSPA1A (SEQ ID NO: 52)	TGTTGATATTGTTTGGTTA (SEQ ID NO: 856)

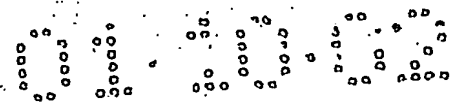
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No:	Gene	Oligo:
419	HSPA1A (SEQ ID NO: 52)	ATGGCGATGTTGATCGTT (SEQ ID NO: 857)
420	HSPA1A (SEQ ID NO: 52)	ATGGTGATGTTGATTGTT (SEQ ID NO: 858)
421	FKBP4 (SEQ ID NO: 53)	TTTATATTTTCGTCGTAGG (SEQ ID NO: 859)
422	FKBP4 (SEQ ID NO: 53)	TTTTTATATTTTGTGTAGG (SEQ ID NO: 860)
423	FKBP4 (SEQ ID NO: 53)	ATGTCGGGAGATCGAT (SEQ ID NO: 861)
424	FKBP4 (SEQ ID NO: 53)	GATGTTGGGAGATTGAT (SEQ ID NO: 862)
425	FKBP4 (SEQ ID NO: 53)	AGGTCGTCGGGGAGAT (SEQ ID NO: 863)
426	FKBP4 (SEQ ID NO: 53)	AGGTTGTTGGGGAGAT (SEQ ID NO: 864)
427	FKBP4 (SEQ ID NO: 53)	AGATTTTCGAGTTTCGAG (SEQ ID NO: 865)
428	FKBP4 (SEQ ID NO: 53)	ATTTTGAGTTTGAGGGT (SEQ ID NO: 866)
429	ESR2 (SEQ ID NO: 54)	ATTTTCGAGGATTACGTT (SEQ ID NO: 867)
430	ESR2 (SEQ ID NO: 54)	ATTTTGAGGATTATGTTTT (SEQ ID NO: 868)
431	ESR2 (SEQ ID NO: 54)	AGATGGCGTTTTTCGTA (SEQ ID NO: 869)
432	ESR2 (SEQ ID NO: 54)	TAGATGGTGTTTTTTGTA (SEQ ID NO: 870)
433	ESR2 (SEQ ID NO: 54)	ATTTTCGAATCGATTTTT (SEQ ID NO: 871)
434	ESR2 (SEQ ID NO: 54)	GGAGTATTTTTGAATTGAT (SEQ ID NO: 872)
435	ESR2 (SEQ ID NO: 54)	AGTTCGACGGTTTTAG (SEQ ID NO: 873)
436	ESR2 (SEQ ID NO: 54)	AGGGAGTTTGATGGTT (SEQ ID NO: 874)
437	ESR2 (SEQ ID NO: 54)	AGTTTACGTGATCGAG (SEQ ID NO: 875)
438	ESR2 (SEQ ID NO: 54)	AGTTTATGTGATTGAGTT (SEQ ID NO: 876)
439	IGF1 (SEQ ID NO: 55)	AGATTATGTCGAGTTGT (SEQ ID NO: 877)
440	IGF1 (SEQ ID NO: 55)	AAGATTATGTTGAGTTGT (SEQ ID NO: 878)
441	IGF1 (SEQ ID NO: 55)	TAGTATTCGGGTGATTT (SEQ ID NO: 879)
442	IGF1 (SEQ ID NO: 55)	AGATAGTATTTGGGTGAT (SEQ ID NO: 880)
443	IGF1 (SEQ ID NO: 55)	AGAAAGGACGATAAGAT (SEQ ID NO: 881)



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No:	Gene	Oligo:
444	IGF1 (SEQ ID NO: 55)	AGAAAGGATGATAAGATT (SEQ ID NO: 882)
445	VTN (SEQ ID NO: 56)	GGTGGTATCGATTGAT (SEQ ID NO: 883)
446	VTN (SEQ ID NO: 56)	TGGTGGTATTGATTGAT (SEQ ID NO: 884)
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448	VTN (SEQ ID NO: 56)	TAGTGATTTGTGGGGA (SEQ ID NO: 886)
449	VTN (SEQ ID NO: 56)	TTATGTCGGAGGATGA (SEQ ID NO: 887)
450	VTN (SEQ ID NO: 56)	ATTATGTTGGAGGATGA (SEQ ID NO: 888)
451	VTN (SEQ ID NO: 56)	ATACGGTTTATGACGAT (SEQ ID NO: 889)
452	VTN (SEQ ID NO: 56)	ATATGGTTTATGATGATGG (SEQ ID NO: 890)
453	CTSL (SEQ ID NO: 57)	GGGTCGGGTGGACGAA (SEQ ID NO: 891)
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455	CTSL (SEQ ID NO: 57)	TAAACGTGGGGTCGGG (SEQ ID NO: 893)
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458	CTSL (SEQ ID NO: 57)	TTGGAGTGTTTGAGTTA (SEQ ID NO: 896)
459	TGFB3 (SEQ ID NO: 58)	AGGACGAAGAAGCGGA (SEQ ID NO: 897)
460	TGFB3 (SEQ ID NO: 58)	AGGATGAAGAAGTGGA (SEQ ID NO: 898)
461	TGFB3 (SEQ ID NO: 58)	TAGGAAGCGTTGGTAA (SEQ ID NO: 899)
462	TGFB3 (SEQ ID NO: 58)	TTAGGAAGTGTTGGTA (SEQ ID NO: 900)
463	TGFB3 (SEQ ID NO: 58)	TAGTAGACGTGTAGAAG (SEQ ID NO: 901)
464	TGFB3 (SEQ ID NO: 58)	AGTAGATGTGTAGAAGG (SEQ ID NO: 902)
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466	TGFB3 (SEQ ID NO: 58)	AGGTTGATTGTGGTAAA (SEQ ID NO: 904)
467	MAPKAPK5 (SEQ ID NO: 59)	AGTAGTCGTTTTTCGGGA (SEQ ID NO: 905)
468	MAPKAPK5 (SEQ ID NO: 59)	AGTAGTTGTTTTTTGGGA (SEQ ID NO: 906)



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No:	Gene	Oligo:
469	MAPKAPK5 (SEQ ID NO: 59)	AATTCGGTAGTCGATG (SEQ ID NO: 907)
470	MAPKAPK5 (SEQ ID NO: 59)	ATTTGGTAGTTGATGGA (SEQ ID NO: 908)
471	MAPKAPK5 (SEQ ID NO: 59)	ATTCGGGGGAGGTTCGAA (SEQ ID NO: 909)
472	MAPKAPK5 (SEQ ID NO: 59)	ATTTGGGGGAGGTTGAA (SEQ ID NO: 910)
473	MAPKAPK5 (SEQ ID NO: 59)	ATTTACGGAATTCGGG (SEQ ID NO: 911)
474	MAPKAPK5 (SEQ ID NO: 59)	TTATGGAATTTGGGGG (SEQ ID NO: 912)
475	PCAF (SEQ ID NO: 60)	GAGCGGTAGGTGTCGAA (SEQ ID NO: 913)
476	PCAF (SEQ ID NO: 60)	GAGTGGTAGGTGTTGAA (SEQ ID NO: 914)
477	PCAF (SEQ ID NO: 60)	TAAGATTTTCGCGGGTA (SEQ ID NO: 915)
478	PCAF (SEQ ID NO: 60)	TGTAAGATTTTGTGGGTA (SEQ ID NO: 916)
479	PCAF (SEQ ID NO: 60)	AGTTCGTAGTTTCGAG (SEQ ID NO: 917)
480	PCAF (SEQ ID NO: 60)	GTTTGTAGTTTGTAGGA (SEQ ID NO: 918)
481	PCAF (SEQ ID NO: 60)	TAGGGCGCGGAGTAGA (SEQ ID NO: 919)
482	PCAF (SEQ ID NO: 60)	TAGGGTGTGGAGTAGA (SEQ ID NO: 920)
483	NCOA3 (SEQ ID NO: 61)	GATGATATTTTCGGGATTA (SEQ ID NO: 921)
484	NCOA3 (SEQ ID NO: 61)	GATGATATTTTGGGATTAA (SEQ ID NO: 922)
485	NCOA3 (SEQ ID NO: 61)	ATAGTTTCGTGGTTTTCGAT (SEQ ID NO: 923)
486	NCOA3 (SEQ ID NO: 61)	ATAGTTTTGTGGTTTTTGAT (SEQ ID NO: 924)
487	NCOA3 (SEQ ID NO: 61)	TTTAGTTCGTTTATTTGAA (SEQ ID NO: 925)
488	NCOA3 (SEQ ID NO: 61)	TTTAGTTTGTTTATTTGAAA (SEQ ID NO: 926)
489	NCOA3 (SEQ ID NO: 61)	AGGATTCGTGTTTGGTA (SEQ ID NO: 927)
490	NCOA3 (SEQ ID NO: 61)	AGGATTTGTGTTTGGTA (SEQ ID NO: 928)
491	PRKCD (SEQ ID NO: 62)	ATTTATTTTTCGTTGTAGG (SEQ ID NO: 929)
492	PRKCD (SEQ ID NO: 62)	TATTTATTTTGTGTTGTAGG (SEQ ID NO: 930)
493	PRKCD (SEQ ID NO: 62)	TTTCGGAAACGGGAAT (SEQ ID NO: 931)

No:	Gene	Oligo:
494	PRKCD (SEQ ID NO: 62)	TAGTTTTGGAAATGGGA (SEQ ID NO: 932)
495	PRKCD (SEQ ID NO: 62)	GGACGGAGTTATCGGT (SEQ ID NO: 933)
496	PRKCD (SEQ ID NO: 62)	GGATGGAGTTATTGGTA (SEQ ID NO: 934)
497	PRKCD (SEQ ID NO: 62)	GTTTAGCGGAGGGATA (SEQ ID NO: 935)
498	PRKCD (SEQ ID NO: 62)	TGTTTAGTGGAGGGAT (SEQ ID NO: 936)
499	PTGS2 (SEQ ID NO: 63)	TTTATCGGGTTTACGTAATT (SEQ ID NO: 955)
500	PTGS2 (SEQ ID NO: 63)	TTTATTGGGTTTATGTAATT (SEQ ID NO: 956)
501	PTGS2 (SEQ ID NO: 63)	GTACGAAAAGGCGGAAAG (SEQ ID NO: 957)
502	PTGS2 (SEQ ID NO: 63)	GTATGAAAAGGTGGAAAG (SEQ ID NO: 958)
503	PTGS2 (SEQ ID NO: 63)	ATTGGTTTTTCGGAAGCGTT (SEQ ID NO: 937)
504	PTGS2 (SEQ ID NO: 63)	ATTGGTTTTTGGAAGTGTT (SEQ ID NO: 938)
505	PTGS2 (SEQ ID NO: 63)	AAGCGTTCGGGTAAAGAT (SEQ ID NO: 939)
506	PTGS2 (SEQ ID NO: 63)	AAGTGTTTGGGTAAAGAT (SEQ ID NO: 940)

E30095
Epigenomics AG

Patent Claims

1. A method for predicting the responsiveness of a subject with a cell proliferative disorder of the breast tissues to a therapy comprising the drug tamoxifen, comprising a) analysing the methylation pattern of a target nucleic acid comprising one or a combination of the genes taken from the group consisting of FGFR1, PSA, CGA, PTGS2, MSMB, TP53 and CYP2D6 and/or their regulatory regions by contacting at least one of said target nucleic acids in a biological sample obtained from said subject with at least one reagent or a series of reagents, wherein said reagent or series of reagents, distinguishes between methylated and non methylated CpG dinucleotides within the target nucleic acid, and b) comparing the methylation pattern of the at least one target nucleic acid with the methylation pattern of said target nucleic acid from an untreated subject.
2. A method according to Claim 1, wherein said target nucleic acid or acids comprise essentially one or more sequences from the group consisting of SEQ ID NOS: 15, 31, 32, 63, 40, 9 and 33 and sequences complementary thereto.
3. A method according to Claims 1 or 2, wherein said cell proliferative disorder of the breast tissue is selected from the group consisting of ductal carcinoma *in situ*, lobular carcinoma, colloid carcinoma, tubular carcinoma, medullary carcinoma, metaplastic carcinoma, intra-ductal carcinoma *in situ*, lobular carcinoma *in situ* and papillary carcinoma *in situ*.
4. A method according to Claims 1 to 3, wherein said tamoxifen therapy is an adjuvant treatment.
5. A method according to Claim 5, wherein said subjects are estrogen and/or progesterone receptor positive.
6. A method according to Claims 4 or 5, wherein the target nucleic acid is selected from the genes PTGS2, MSMB, TP53 and CYP2D6 and/or their regulatory regions and combinations thereof.

7. A method according to Claims 1 to 3, wherein said tamoxifen therapy is for the treatment of a relapse or metastatic cell proliferative disorder of the breast tissues.
8. A method according to claim 7, wherein said subjects have not received a chemotherapy.
9. A method according to Claims 7 or 8, wherein the target nucleic acid is selected from the genes FGFR1, PSA and CGA and/or their regulatory regions and combinations thereof.
10. A nucleic acid molecule consisting essentially of a sequence at least 18 bases in length according to one of the sequences taken from the group consisting of SEQ ID NOS: 109, 110, 234, 235, 141, 142 267, 268, 143, 144, 269, 270, 205, 206, 331, 332, 159, 160, 285, 286, 97, 98, 223, 224, 145, 146, 271 and 272 and sequences complementary thereto.
11. An oligomer, in particular an oligonucleotide or peptide nucleic acid (PNA)-oligomer, said oligomer consisting essentially of at least one base sequence having a length of at least 10 nucleotides which hybridises to or is identical to one of the nucleic acid sequences according to SEQ ID NOS:15, 31, 32, 63, 40, 9, 33, 109, 110, 234, 235, 141, 142 267, 268, 143, 144, 269, 270, 205, 206, 331, 332, 159, 160, 285, 286, 97, 98, 223, 224, 145, 146, 271 and 272.
12. The oligomer as recited in Claim 11, wherein the base sequence includes at least one CpG dinucleotide.
13. The oligomer as recited in Claim 12, wherein the cytosine of the CpG dinucleotide is located approximately in the middle third of the oligomer.
14. A set of oligomers, comprising at least two oligomers according to any of claims 11 to 13.
15. A set of oligomers as recited in Claim 14, comprising oligomers for detecting the methylation state of all CpG dinucleotides within the group of SEQ ID NOS:15, 31, 32, 63, 40, 9, 33, 109, 110, 234, 235, 141, 142 267, 268, 143, 144, 269, 270, 205, 206, 331, 332, 159, 160, 285, 286, 97, 98, 223, 224, 145, 146, 271 and 272 and sequences complementary thereto.

16. A set of at least two oligonucleotides as recited in one of Claims 11 through 15, which is used as primer oligonucleotides for the amplification of nucleic acid sequences of one of SEQ ID NOS:15, 31, 32, 63, 40, 9, 33, 109, 110, 234, 235, 141, 142 267, 268, 143, 144, 269, 270, 205, 206, 331, 332, 159, 160, 285, 286, 97, 98, 223, 224, 145, 146, 271 and 272 and sequences complementary thereto.
17. A set of oligonucleotides as recited in one of Claims 14 through 10, characterised in that at least one oligonucleotide is bound to a solid phase.
18. Use of a set of oligonucleotides comprising at least three of the oligomers according to any of claims 11 through 17 for detecting the cytosine methylation state and/or single nucleotide polymorphisms (SNPs) within the sequences taken from the group of SEQ ID NOS:15, 31, 32, 63, 40, 9, 33, 109, 110, 234, 235, 141, 142 267, 268, 143, 144, 269, 270, 205, 206, 331, 332, 159, 160, 285, 286, 97, 98, 223, 224, 145, 146, 271 and 272 and sequences complementary thereto.
19. A method for manufacturing an arrangement of different oligomers (array) fixed to a carrier material for predicting breast cell proliferative disorders' response to tamoxifen treatment by analysis of the methylation state of any of the CpG dinucleotides of the group SEQ ID NOS:15, 31, 32, 63, 40, 9, 33, 109, 110, 234, 235, 141, 142 267, 268, 143, 144, 269, 270, 205, 206, 331, 332, 159, 160, 285, 286, 97, 98, 223, 224, 145, 146, 271 and 272 wherein at least one oligomer according to any of the claims 11 through 17 is coupled to a solid phase.
20. An arrangement of different oligomers (array) obtainable according to claim 19.
21. An array of different oligonucleotide- and/or PNA-oligomer sequences as recited in Claim 20, characterised in that said oligonucleotides are arranged on a plane solid phase in the form of a rectangular or hexagonal lattice.
22. The array as recited in any of the Claims 20 or 21, characterised in that the solid phase surface is composed of silicon, glass, polystyrene, aluminium, steel, iron, copper, nickel, silver, or gold.

23. A DNA- and/or PNA-array for predicting breast cell proliferative disorders' response to tamoxifen treatment by analysis of the methylation state of any of the CpG dinucleotides within the sequences taken from the group of SEQ ID NOS:15, 31, 32, 63, 40, 9, 33, 109, 110, 234, 235, 141, 142 267, 268, 143, 144, 269, 270, 205, 206, 331, 332, 159, 160, 285, 286, 97, 98, 223, 224, 145, 146, 271 and 272 comprising at least one nucleic acid according to one of the preceding claims.
24. A method according to any one of Claims 1 to 3 comprising the following steps:
 - obtaining a biological sample containing genomic DNA, from a subject,
 - b) extracting the genomic DNA from said sample,
 - c) converting cytosine bases in the genomic DNA sample which are unmethylated at the 5-position, by chemical treatment, to uracil or another base which is dissimilar to cytosine in terms of base pairing behaviour;
 - d) amplifying at least one fragment of the pretreated genomic DNA, wherein said fragments comprise one or more sequences selected from the group consisting of SEQ ID NOS: 109, 110, 234, 235, 141, 142 267, 268, 143, 144, 269, 270, 205, 206, 331, 332, 159, 160, 285, 286, 97, 98, 223, 224, 145, 146, 271 and 272 and sequences complementary thereto,
 - e) determining the methylation status of the genomic CpG dinucleotides by analysis of the amplificate nucleic acids.
25. The method according to Claim 24 or 6, characterised in that Step e) is carried out by means of hybridisation of at least one oligonucleotide according to SEQ ID NO: 955, 956, 956, 957, 957, 958, 958 - 964.
26. The method according to Claim 24 or 9, characterised in that Step e) is carried out by means of hybridisation of at least one oligonucleotide according to SEQ ID NO: 941 - 954.
27. The method as recited in Claim 24, characterised in that Step e) is carried out by means of hybridisation of at least one oligonucleotide according to Claims 11 through 17.

28. The method as recited in Claim 24, characterised in that Step e) is carried out by means of hybridisation of at least one oligonucleotide according to Claims 11 through 17 and extension of said hybridised oligonucleotide(s) by means of at least one nucleotide base.
29. The method as recited in Claim 24, characterised in that Step e) is carried out by means of sequencing.
30. The method as recited in Claim 24, characterised in that Step d) is carried out using methylation specific primers.
31. The method as recited in Claim 24, characterised in that Step e) is carried out by means of a combination of at least two of the methods described in Claims 27 through 30.
32. The method as recited in Claim 24, characterised in that the treatment is carried out by means of a solution of a bisulfite, hydrogen sulfite or disulfite.
33. A method according to any one of Claims 1 to 3 comprising the following steps:
 - a) obtaining a biological sample containing genomic DNA, from a subject,
 - b) extracting the genomic DNA from said sample,
 - c) digesting the genomic DNA comprising one or more of the sequences from the group consisting of SEQ ID NO:15, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:63, SEQ ID NO:40, SEQ ID NO:9 and SEQ ID NO:33 and sequences complementary thereto with one or more methylation sensitive restriction enzymes, and
 - d) determining of the DNA fragments generated in the digest of step c).
34. The method according to Claim 33 or 6, characterised in that the target sequence or sequences digested in Step C comprises one or more sequences from the group consisting of SEQ ID NOS: 72-78
35. The method according to Claim 33 or 9, characterised in that the target sequence or sequences digested in Step C comprises one or more sequences from the group consisting of SEQ ID NOS: 64-71.

36. A method according to one of Claims 33 to 35, wherein the DNA digest is amplified prior to Step d).
37. The method as recited in one of the Claims 24 through 32 and 36 characterised in that more than ten different fragments having a length of 100 - 200 base pairs are amplified.
38. The method as recited in one of Claims 24 through 32, 36 and 37 characterised in that the amplification of several DNA segments is carried out in one reaction vessel.
39. The method as recited in one of the Claims 24 through 32 and 36 through 38, characterised in that the polymerase is a heat-resistant DNA polymerase.
40. The method as recited in one of the Claims 24 through 32 and 36 through 39, characterised in that the amplification is carried out by means of the polymerase chain reaction (PCR).
41. The method as recited in one of the 24 through 32 and 36 through 40, characterised in that the amplicates carry detectable labels.
42. The method according to Claim 41 wherein said labels are fluorescence labels, radionuclides and/or detachable molecule fragments having a typical mass which can be detected in a mass spectrometer.
43. The method as recited in one of the Claims 24 through 32, characterised in that the amplicates or fragments of the amplicates are detected in the mass spectrometer.
44. The method as recited in one of the Claims 42 and/or 43, characterised in that the produced fragments have a single positive or negative net charge for better detectability in the mass spectrometer.
45. The method as recited in one of Claims 41 through 44, characterised in that detection is carried out and visualised by means of matrix assisted laser desorption/ionisation mass spectrometry (MALDI) or using electron spray mass spectrometry (ESI).

46. The method as recited in one of the Claims 24 through 41, characterised in that the genomic DNA is obtained from cells or cellular components which contain DNA, sources of DNA comprising, for example, cell lines, histological slides, biopsies, tissue embedded in paraffin, breast tissues, blood, plasma, lymphatic fluid, lymphatic tissue, duct cells, ductal lavage fluid, nipple aspiration fluid, bone marrow and combinations thereof.
47. A kit comprising a bisulfite (= disulfite, hydrogen sulfite) reagent as well as oligonucleotides and/or PNA-oligomers according to one of the Claims 11 through 17.
48. A kit according to claim 47, further comprising standard reagents for performing a methylation assay from the group consisting of MS-SNuPE, MSP, Methyl light, Heavy Methyl, nucleic acid sequencing and combinations thereof.
49. The use of a method according to one of claims 1 through 9, 19, and 24 to 46, a nucleic acid according to Claim 10, of an oligonucleotide or PNA-oligomer according to one of the Claims 11 through 17, of a kit according to Claim 43 or 44, of an array according to one of the Claims 21 through 23 or of a set of oligonucleotides according to one of claims 14 through 17 for the characterisation, classification and/or differentiation of breast cell proliferative disorders, or the predisposition to cell proliferative disorders.

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E30095
Epigenomics AG

Abstract

The present invention relates to modified and genomic sequences, to oligonucleotides and/or PNA-oligomers for detecting the cytosine methylation state of genomic DNA, as well as to a method for predicting the response of a subject with a cell proliferative disorder of the breast tissues, to treatment with the drug Tamoxifen.

Figure 1

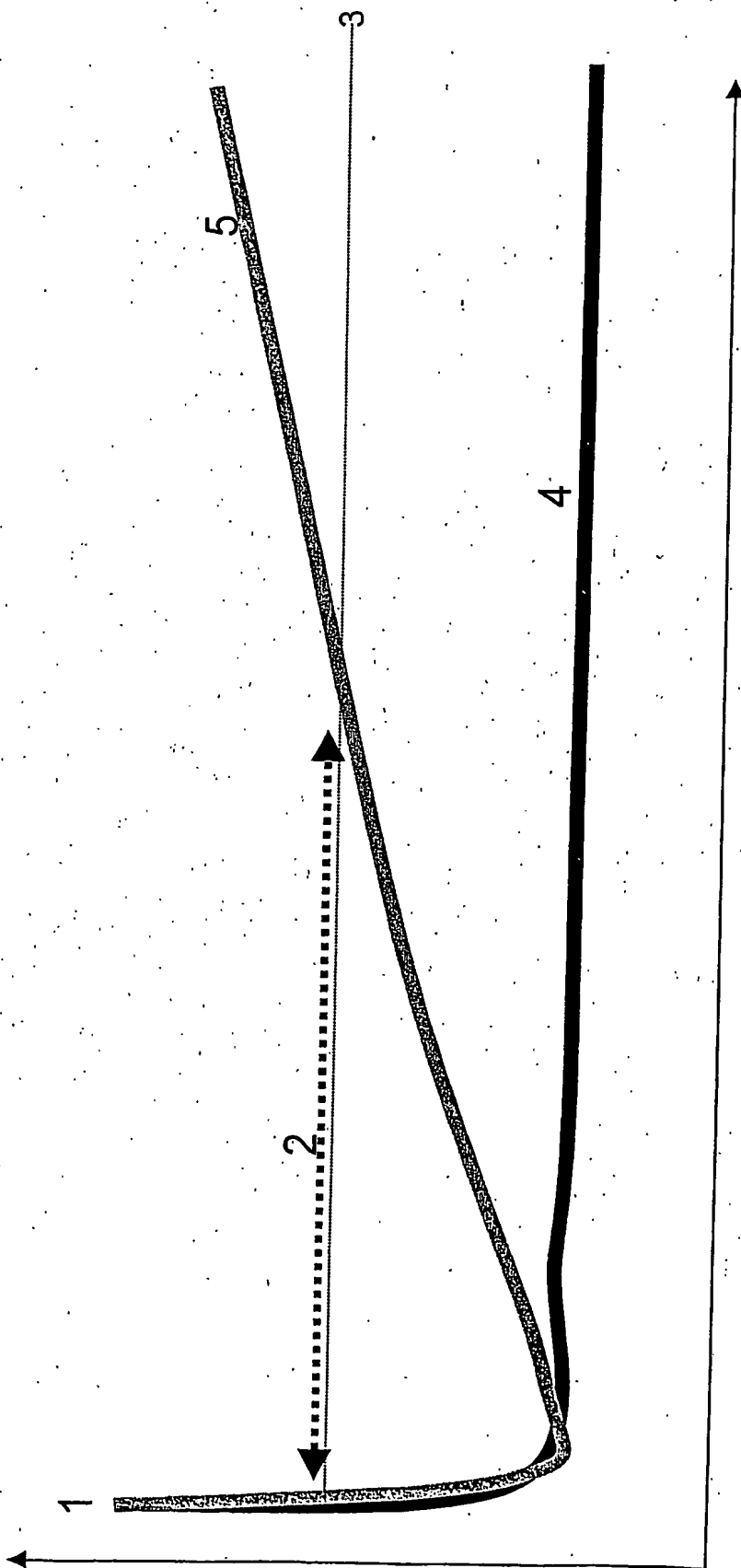
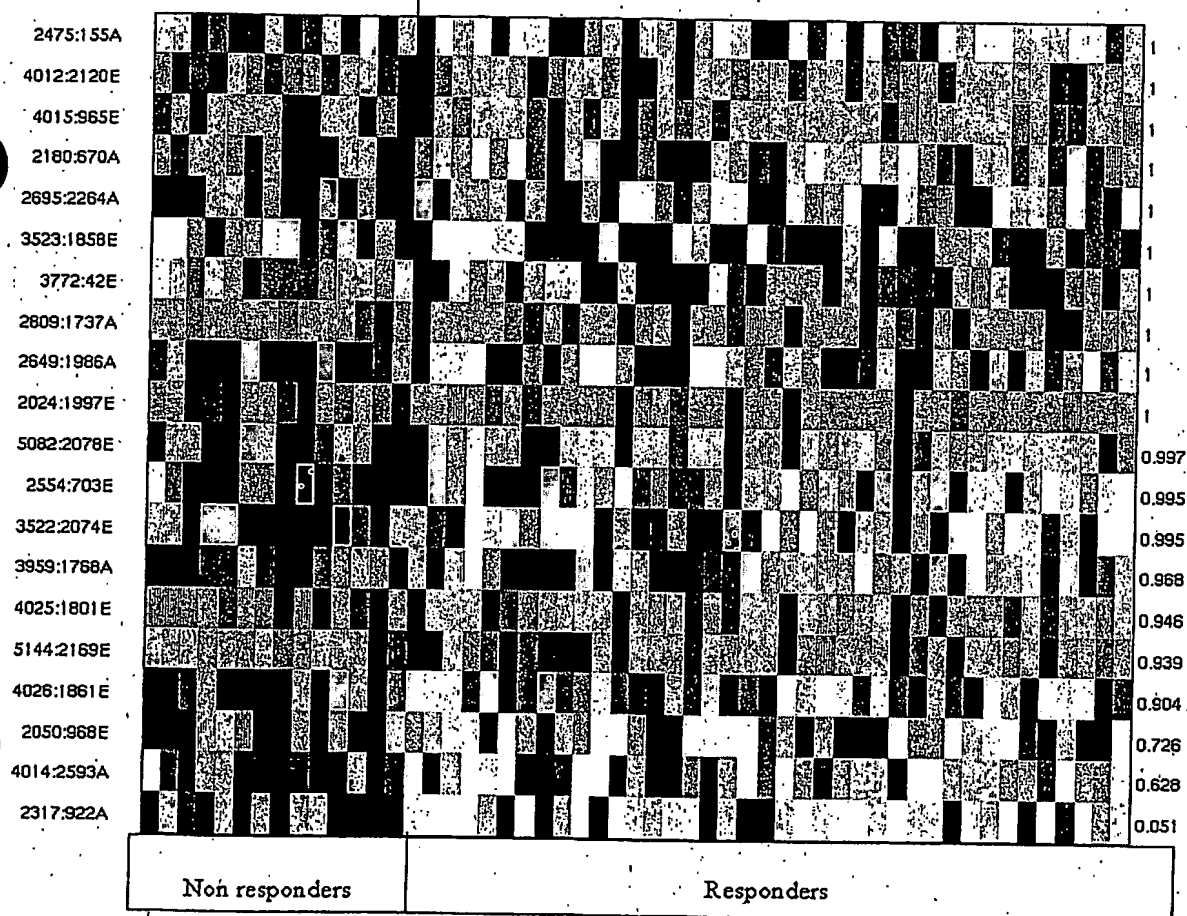


Figure 3



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Figure 4

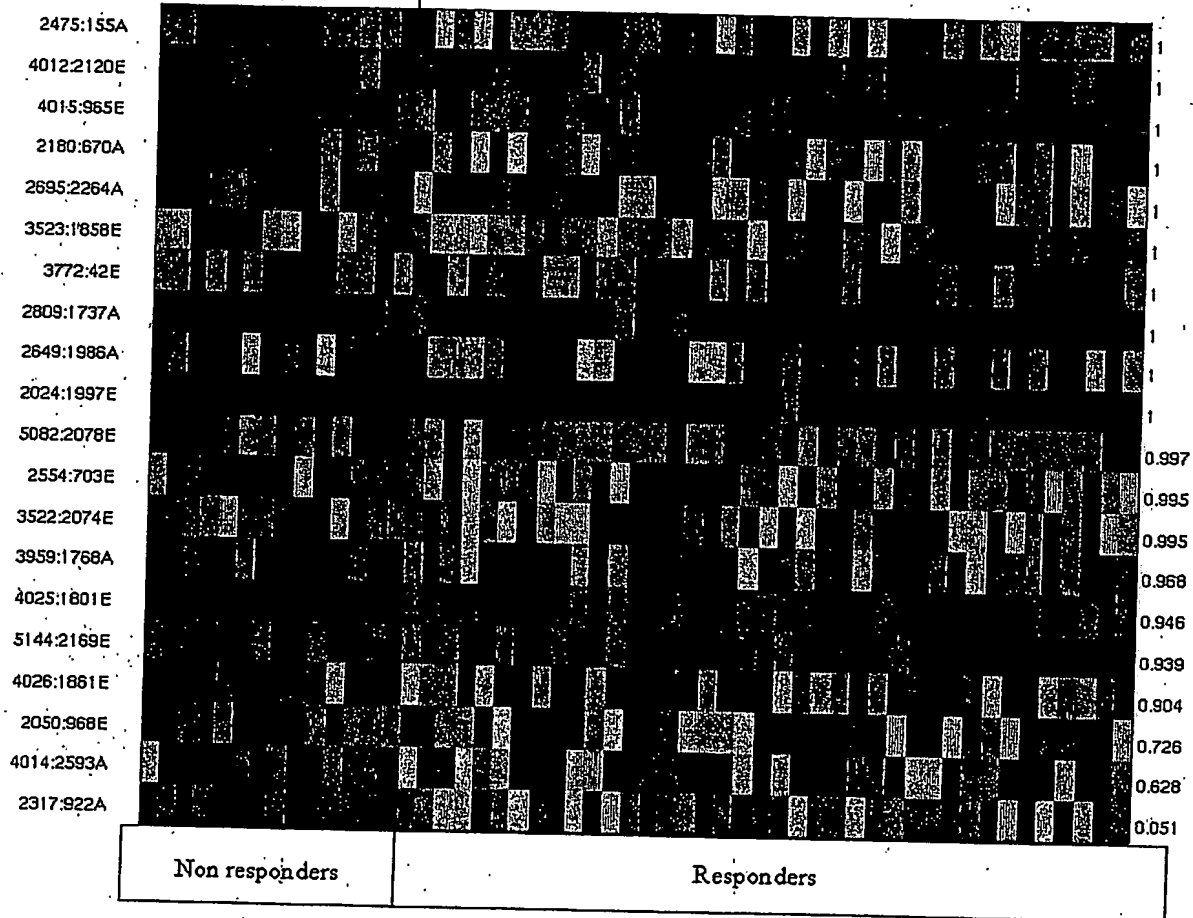
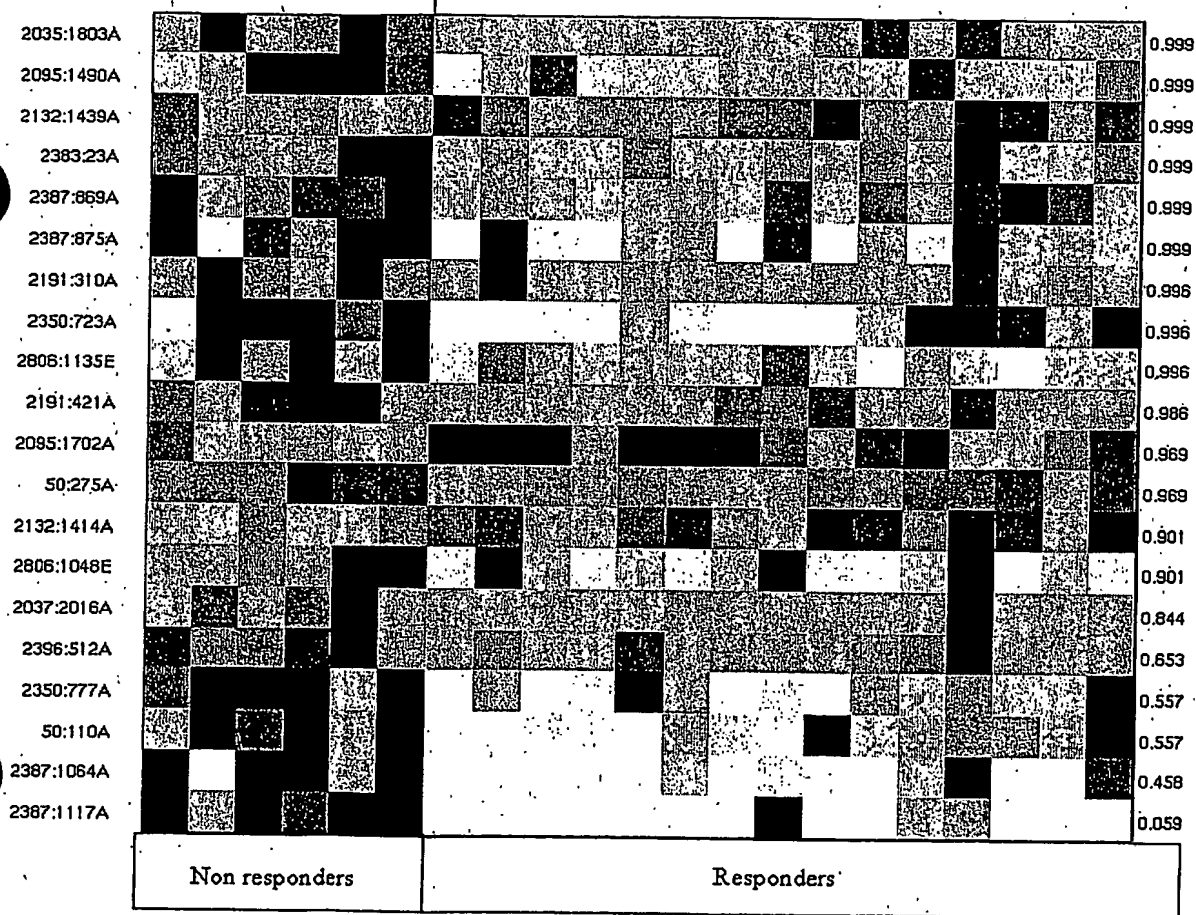


Figure 5



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Figure 6

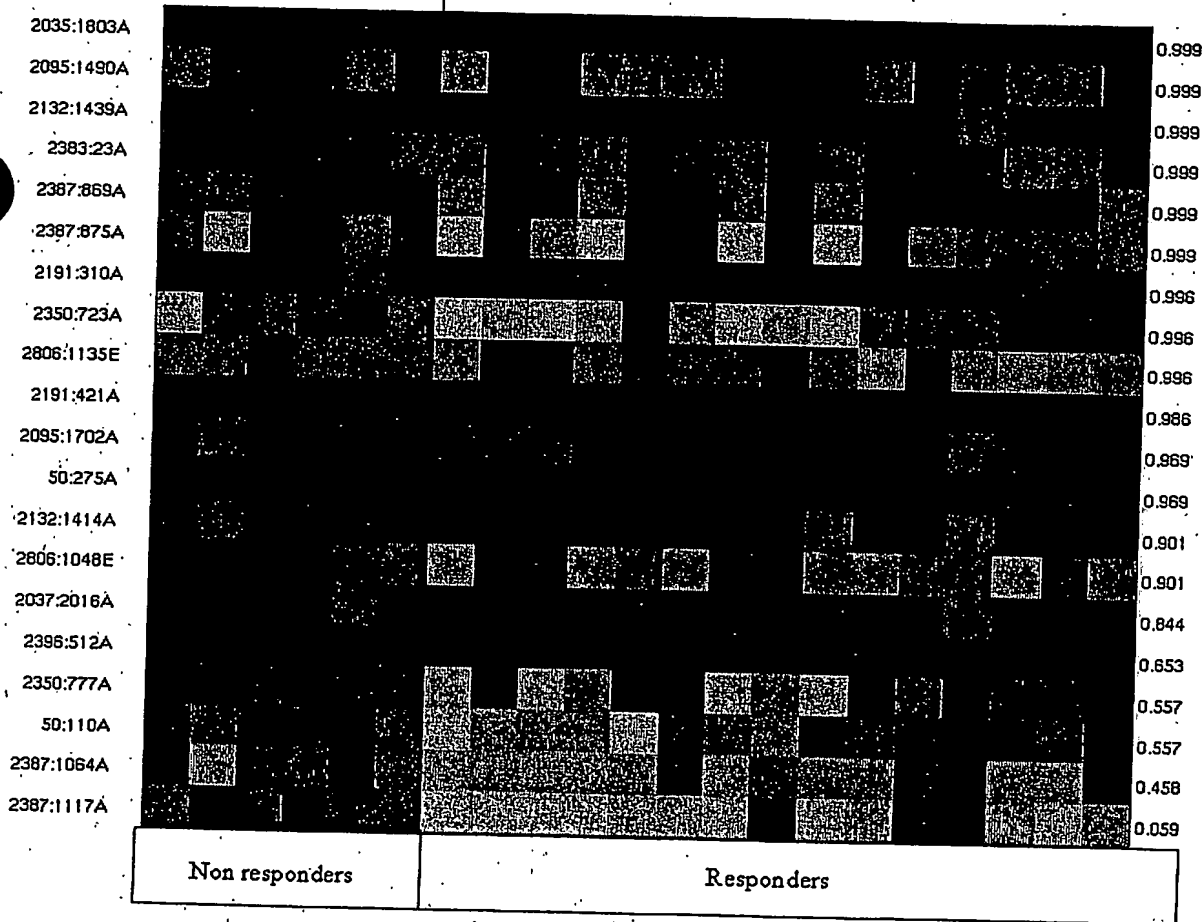
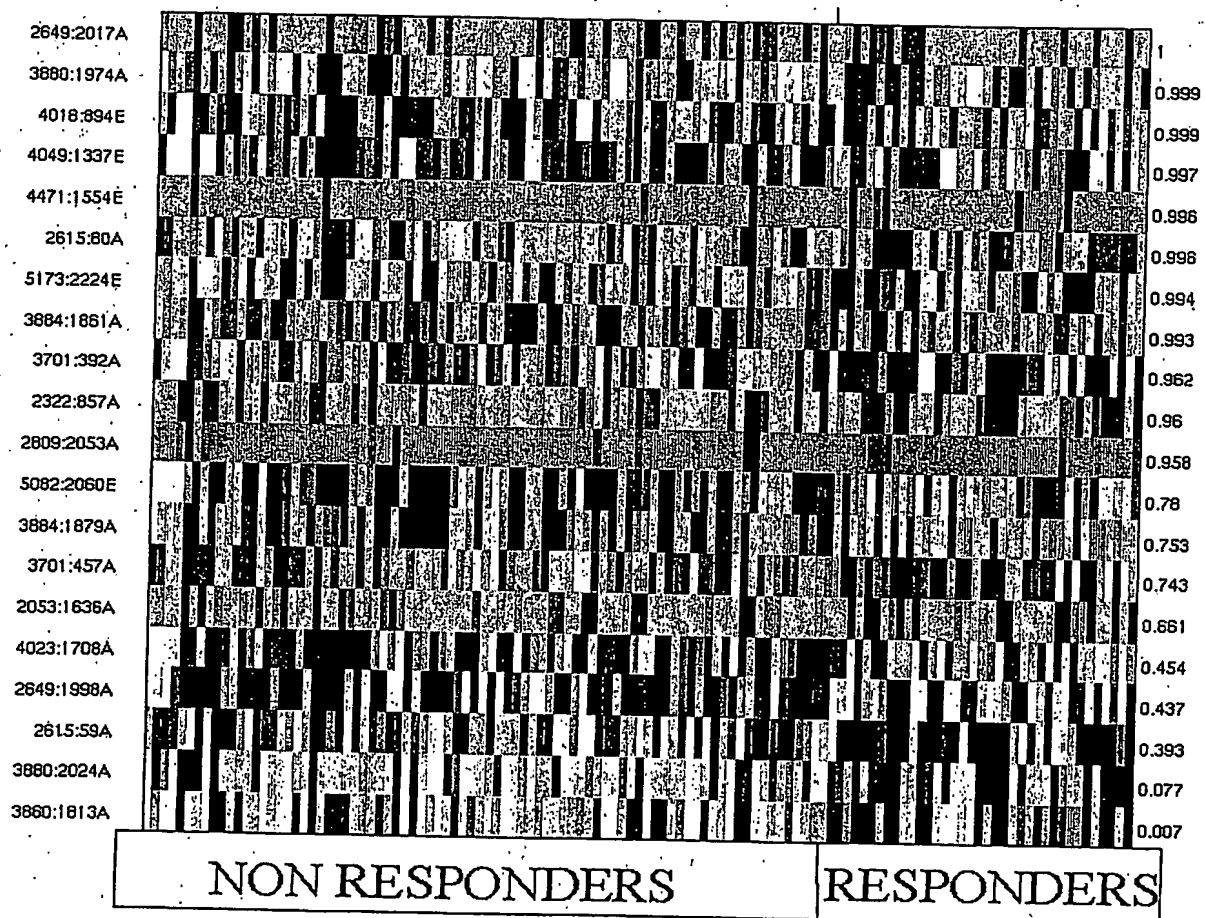


Figure 7

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Figure 8

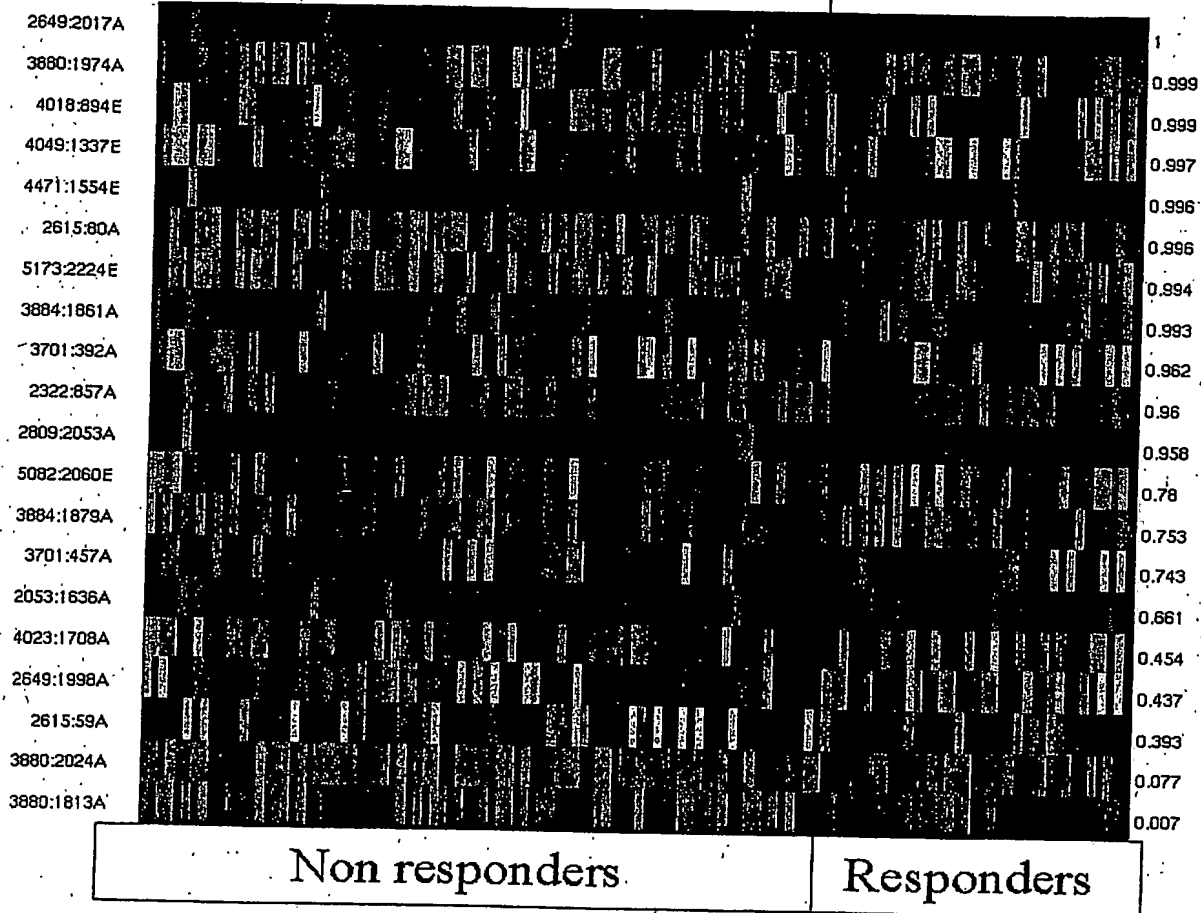
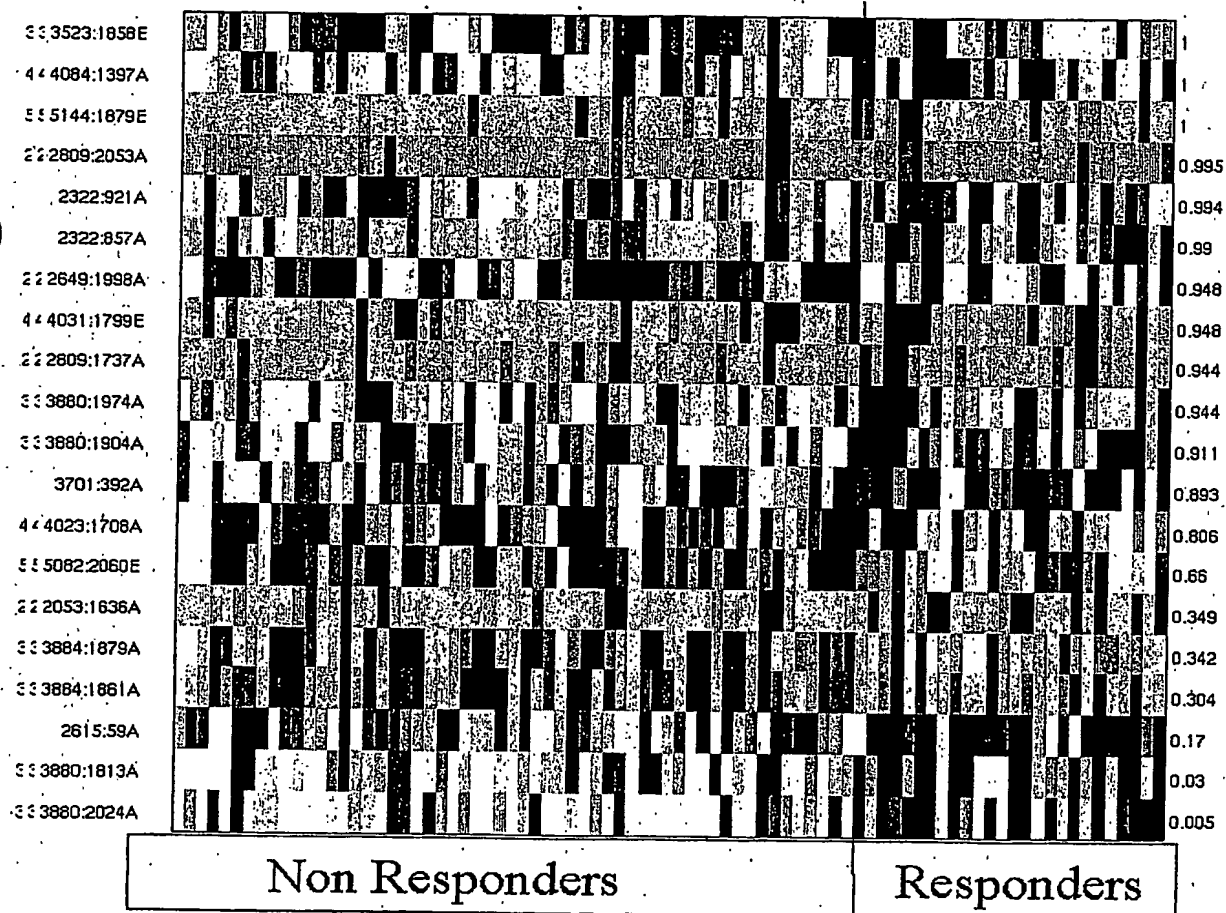


Figure 9



Sequence listing

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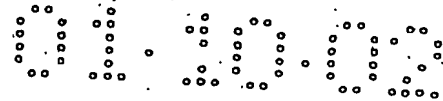
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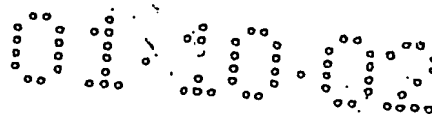
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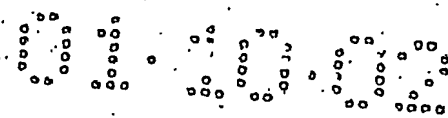
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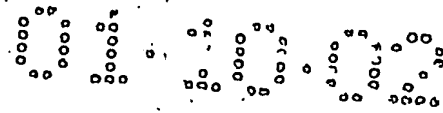
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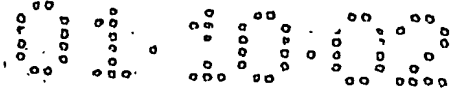
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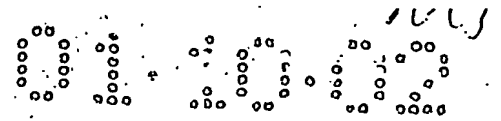
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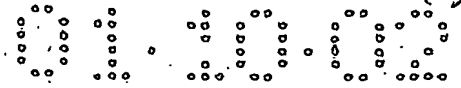
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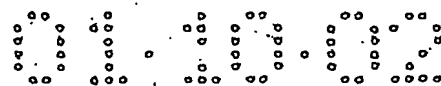
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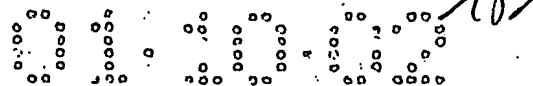
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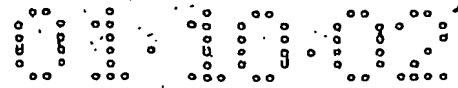
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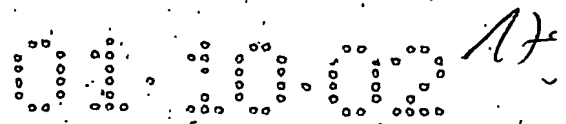
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13> Homo Sapiens

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<210> 84

<211> 3862

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 84

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<10> 85

<211> 2358

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<210> 87

<211> 2501

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 87

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<210> 88

<211> 2501

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 88

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<210> 89

<211> 3647

<213> Artificial Sequence

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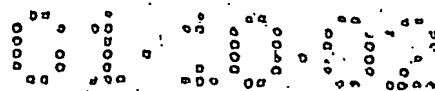
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<211> 5087

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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 <213> Artificial Sequence

<220>
 <223> chemically treated genomic DNA (Homo sapiens)

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<211> 5034

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<223> chemically treated genomic DNA (Homo sapiens)

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<223> unknown base

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> 112

> 2451

> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 112

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<210> 113

<211> 3780

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 113

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<10> 114

<1> 3780

<12> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 114

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- <211> 2515
- <212> DNA
- <213> Artificial Sequence
- <220>
- <223> chemically treated genomic DNA (Homo sapiens)

<400> 115

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aattttgttt	tttgatttcg	aggtttttgg	tttttatttt	agaattaaat	taaattaaat	2460
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<0> 116

<1> 2515

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 116

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tcgttttttt	attttattttt	gtaatgaatg	tatttttgga	tttttagacga	agtttttaaat	180
atgaagtggg	attgttggtt	gggggttaata	ttcgggggttc	gtcgtttcgt	attaataagg	240
ttaggatac	gatataacg	aagagtgggt	ttaggagcgg	aggtttaata	cgtaaaagaa	300
aaaggagaat	agtttttttt	tttgtagag	agagcgggtt	tcgaaaggaa	aatttttaggt	360
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gtgtatatatt	attgaagttt	taattttaatg	gtttattggt	tatttatgta	ttttttttgg	2460
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<210> 117

<211> 3107

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 117

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aaaaaattag	tcgggtatgg	tggcgggcgt	ttatagtttt	agttattcgg	gaggttgagg	180
taggagaatg	gcgtgagttt	aggaggtaga	gtttgcggtg	agttgagatg	atcgggttat	240
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<210> 118

<211> 3107

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 118

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aatttttggt	aagttaggta	aaatttaaaa	aatattttgt	aatattgttt	ttaacgtaaa	180
aaaagattta	aatttttatt	tttttaattg	tttttttagt	ttttgaatat	tttaataaaa	240
gaatggattg	tatttatata	gttttttaggt	gggtttaagg	ttttggagtt	atattgttaa	300
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<210> 119

<211> 3283

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 119

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<211> 3197

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<211> 3155

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<211> 14491

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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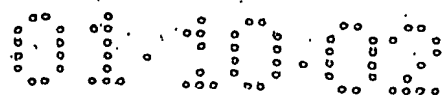
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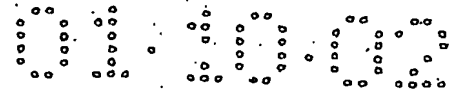
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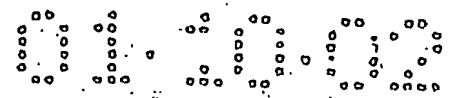
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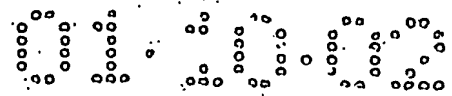


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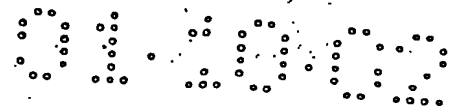
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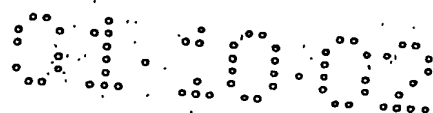
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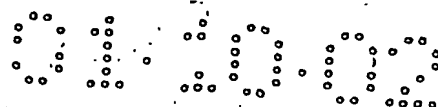
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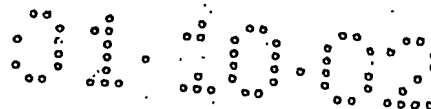
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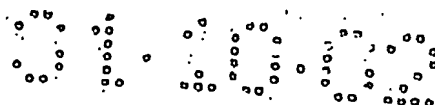
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<220>

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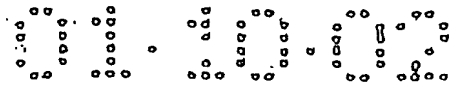
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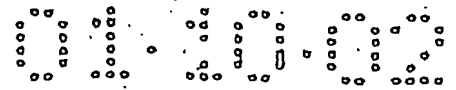
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<210> 145
<211> 2972
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically treated genomic DNA (Homo sapiens)
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<400> 145

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<210> 146

<211> 2972

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 146

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<211> 6101

<212> DNA

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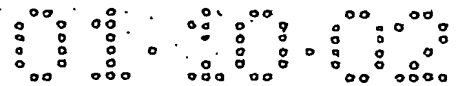
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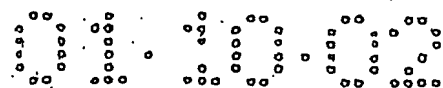
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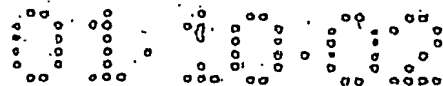
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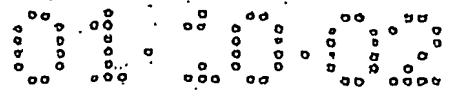
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<211> 2359

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<213> Artificial Sequence

<220>

<221> chemically treated genomic DNA (Homo sapiens)

<222> 151

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<211> 2359

<213> Artificial Sequence

<223> chemically treated genomic DNA (Homo sapiens)

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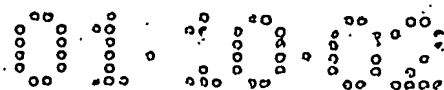
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<213> Artificial Sequence

<223> chemically treated genomic DNA (Homo sapiens)

<400> 153



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<210> 154

<211> 2474

<212> DNA

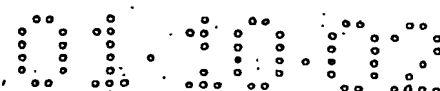
<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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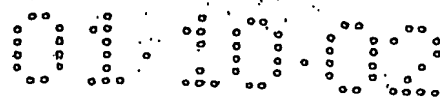
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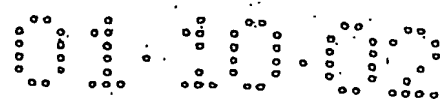
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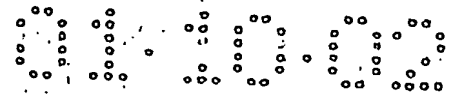
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<211> 1278

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<213> Artificial Sequence

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<223> chemically treated genomic DNA (Homo sapiens)

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<211> 1278

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 162

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<210> 163

<211> 7467

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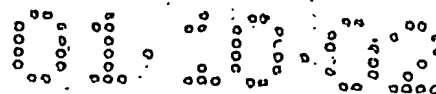
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<220>

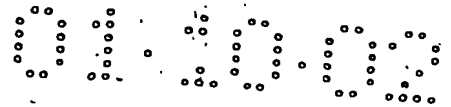
<223> chemically treated genomic DNA (Homo sapiens)

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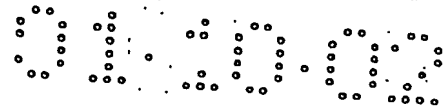
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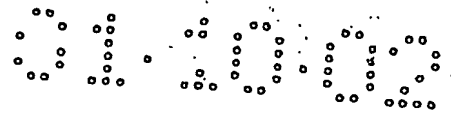
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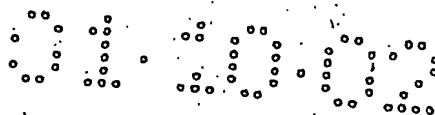
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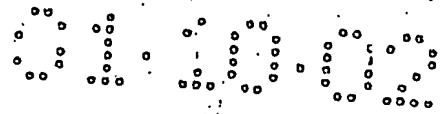
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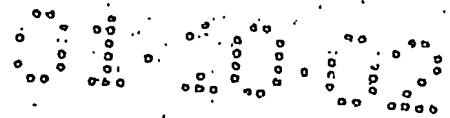
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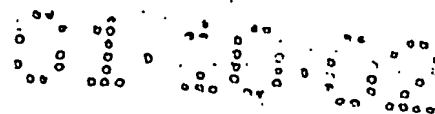


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<213> Artificial Sequence

<220>

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<213> Artificial Sequence

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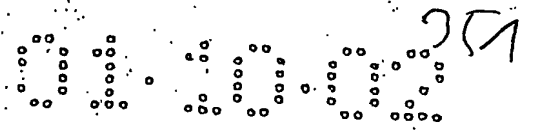
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<222> (1299, 1372, 1784, 3008, 3018)
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<211> 3326

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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359

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<210> 176

<211> 3326

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<211> 9353

<212> DNA

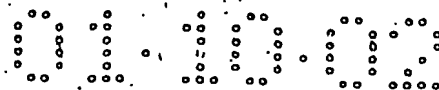
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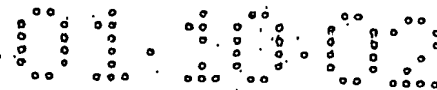
<223> chemically treated genomic DNA (Homo sapiens)

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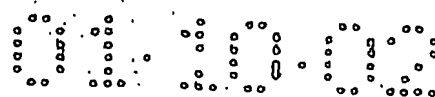
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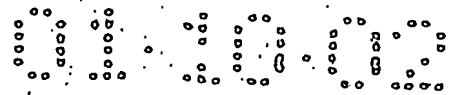
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<213> Artificial Sequence

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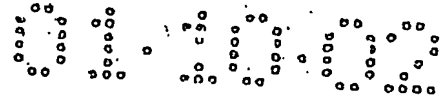
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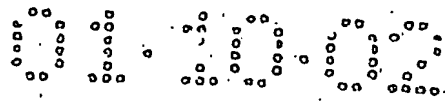
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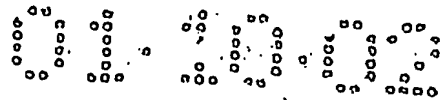
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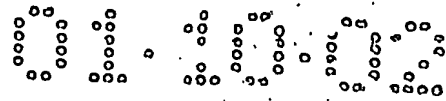
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<220>
 <223> chemically treated genomic DNA (Homo, sapiens)

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<211> 5559

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<211> 3476

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 193

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<211> 3476

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 194

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- <211> 11429
- <212> DNA
- <213> Artificial Sequence
- <220>
- <223> chemically treated genomic DNA (Homo sapiens)

<400> 195

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<220>

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<220>

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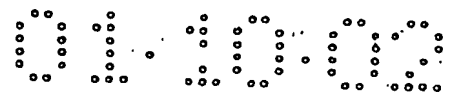
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<210> 207

<211> 2944

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 207

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<210> 208

<211> 2944

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 208

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<210> 209

<211> 3862

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 209

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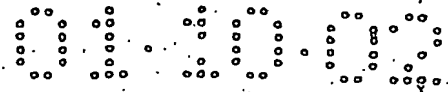
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<210> 210

<211> 3862

<212> DNA



420

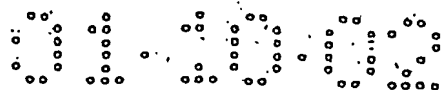
<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 210

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421

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<210> 211
 <211> 2358
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 211

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<210> 212
 <211> 2358

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 212

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ttaaagataa	aattaaagta	ttaagtaatt	taagtttaat	taaaaataga	aattaaagt	300
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<210> 213

<211> 2501

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 213

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<210> 214

<211> 2501

<2> DNA

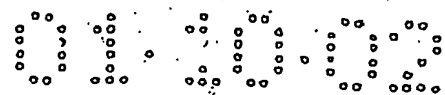
<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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426

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<211> 3647

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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01.20.00

425

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<210> 216

<211> 3647

<2> DNA

<3> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 216

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<211> 3050

<212> DNA

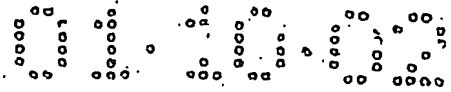
<213> Artificial Sequence

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<223> chemically treated genomic DNA (Homo sapiens)

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421

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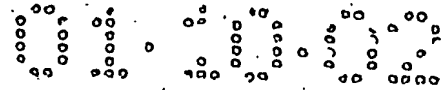
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<220>

<223> chemically treated genomic DNA (Homo sapiens)

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428

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<213> Artificial Sequence

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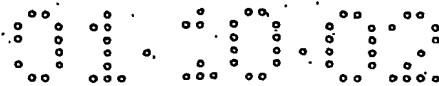
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<213> Artificial Sequence

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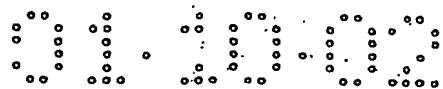
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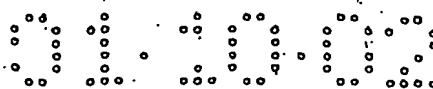
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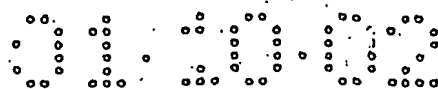
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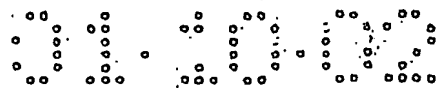
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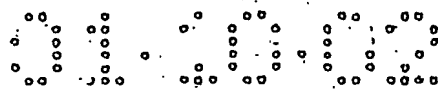


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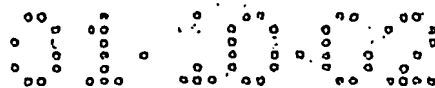
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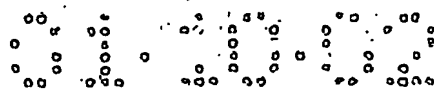
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<211> 3077

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo. sapiens)

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<212> DNA
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<400> 227

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<10> 228

<211> 3814

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 228

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<211> 3223

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<220> 229

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<211> 3223

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<23> chemically treated genomic DNA (Homo sapiens)

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<211> 5034

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<3> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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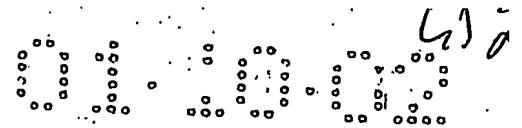
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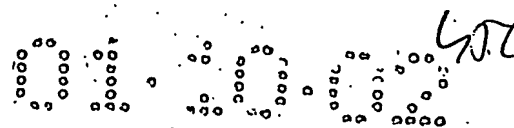
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<210> 237

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<211> 2451
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically treated genomic DNA (Homo sapiens)

<400> 237

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<210> 238
<211> 2451
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically treated genomic DNA (Homo sapiens)

<400> 238

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> 239

> 3780

> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 239

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<210> 240

<211> 3780

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 240

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01.30.00 46

<211> 2515
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 241

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agtagtgTta	tTggaagtaa	aagagaagtt	attTTtaattg	Ttaaagagta	TTTattaaga	300
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gaataagata	ggatattttt	TTTTattatt	aagtaataatt	atattagagg	atttaattaa	420
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aatTTTgttt	TTTgattttg	aggtTTTTTg	TTTTtatttt	agaattaaat	Taaattaaat	2460
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<210> 242
 <211> 2515
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 242

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atgaagtggg	attgtttgtt	gggggttaata	tttgggggtt	gttgttttgt	attaataagg	240
ttaaggatat	gatatatatg	aagagtgggt	ttaggagtg	aggtttaata	tgtaaaagaa	300
aaaggagaat	agtttttttt	tttgtgagag	agagtgggtt	ttgaaaggaa	aatttttaggt	360
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gtgtatatatt	attgaagttt	taatttaaatg	gtttattggg	tatttatgta	ttttttttgg	2460
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<200> 243

<211> 3107

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA. (Homo sapiens)

<400> 243

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aaaaaattag	ttgggtatgg	tggtgggtgt	ttatagtttt	agttatttgg	gaggttgagg	180
taggagaatg	gtgtgagttt	aggaggtaga	gtttgtgggt	agttgagatg	attgggttat	240
tgtatttttag	tttgggtaat	agagttaggt	tttgttttaa	aaaaaaaaaa	aattattata	300
tgatattaag	taatgtggaa	ggtgatttaa	aggggggaaag	gaatatagta	gtgtaaagga	360
aggaggttgt	agatggattt	agaatttttt	ttttattttt	attaggtgaa	agtttgagaa	420
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tttaataatg	ttgataaatt	tgaaaattat	ttgtagatat	tttgtaggtt	ttatttttagg	540
aataatgggt	atttttttgg	gtagttgaag	taaaattaag	tttaatgata	agtaaatata	600
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244

<211> 3107

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

$\langle 400 \rangle$ 2.44

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taattttttt	ttttttttat	tatatgttgt	aatgttttga	attttatatt	agtttgattg	120
aatttttgtt	aagttaggta	aaatttaaaa	aatattttgt	aatattgttt	ttaatgtaaa	180
aaaagattta	aatttttatt	tttttaattg	tttttttagt	ttttgaatat	tttaataaaa	240
gaatggattg	tattttatata	gttttttaggt	gggtttaagg	ttttggagtt	atattgttaa	300
aaatagataa	atagataaaa	agaaatattt	ttaattttta	atttaaaatt	atattttatt	360
atataaaaatt	ttttttgttt	ttaagtttat	tggttaagta	aattatttga	taatgaaaat	420
gtattttata	gagtaattat	gttaaatttt	agtagtggtt	tagaagggtt	gttgtttgaa	480
tgtaatgtgt	gttttttttt	gtagtaaaaag	atttgtttaa	gtttataaga	tgtttggata	540
tttttatttt	gagtaatttt	gataaatatt	gtggattttt	ttagtggatt	tgtgtaaatg	600
gttaaaggaa	aatgttttag	ttttaagatt	gatttttttg	tgttgtgttt	gattttaatt	660

01:30:00

465

ttgtgttttt	tgaggggttg	gttgtgattg	tattttttgt	gttttttggg	ggtttgggtg	720
ttttgggggtg	tttgggttat	tttttgtatg	gtttgatttt	tgtttttttt	gttttaggttt	780
gagttttttt	tttttgggtg	agttttttgga	ttttgagtgt	gggtagggttt	aggagtgaag	840
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aggtagaata	agaaagggtg	tgaaagtagt	gtgttgtttt	ttgtaatttt	ttttttttat	960
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<245> 245

<211> 3283

<212> DNA

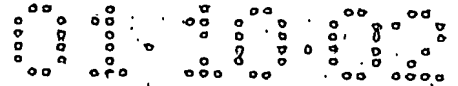
<213> Artificial Sequence

<220>

<223> 'chemically' treated genomic DNA (Homo sapiens)

<400> 245

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gttttaattt	tgaagtttat	ttatggagag	tatatttgtt	aggtgtgtgg	tagatatata	660



46

ggaagtataa	gatgaggtag	tagatttaga	ggtaaagtat	tttttttttt	ttgttttagtg	720
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<210> 246

<211> 3283

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 246

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<210> 247
<211> 3197
<212> DNA
<213> Artificial Sequence

<223> chemically treated genomic DNA (Homo sapiens)

<400> 247

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01:00:00 47

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<210> 248

<211> 3197

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 248

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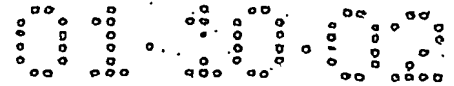
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- <210> 249
- <211> 3155
- <212> DNA
- <213> Artificial Sequence
- <220>
- <223> chemically treated genomic DNA (Homo sapiens)
- <400> 249

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<400> 250



47

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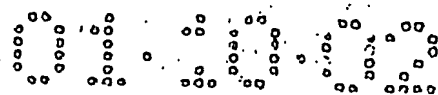
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67

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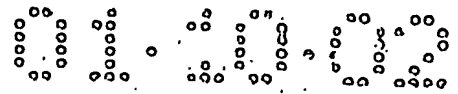
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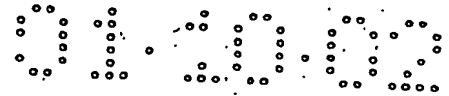
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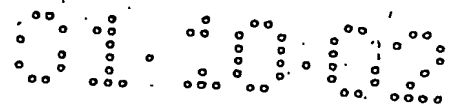


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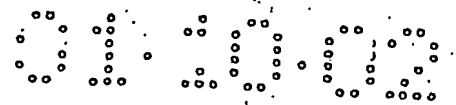
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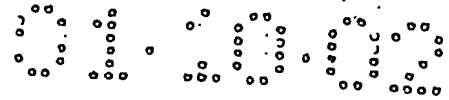
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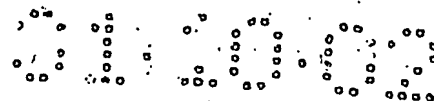
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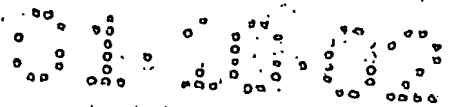
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<223> chemically treated genomic DNA (Homo sapiens)

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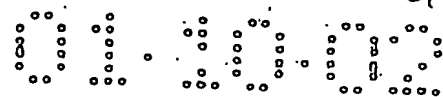
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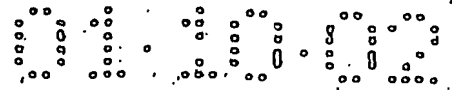
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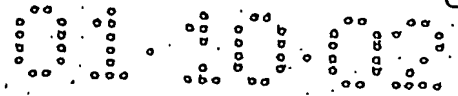
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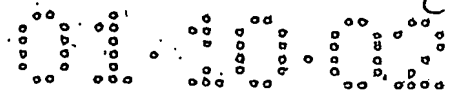
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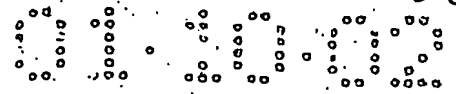
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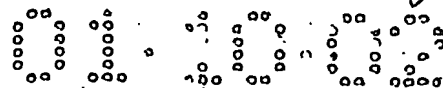
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<213> Artificial Sequence

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<223> chemically treated genomic DNA (Homo sapiens)

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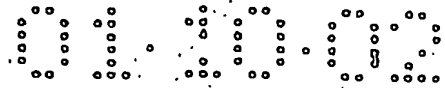
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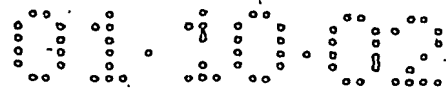
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<213> Artificial Sequence

<220>

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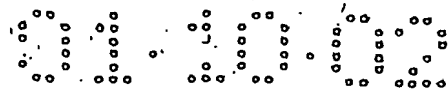
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<212> DNA

<213> Artificial Sequence

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<223> chemically treated genomic DNA (Homo sapiens)

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<212> DNA

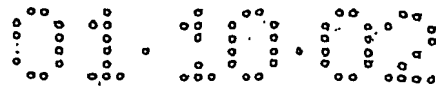
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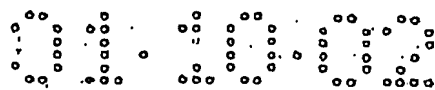
<213> Artificial Sequence

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<223> chemically treated genomic DNA (Homo sapiens)

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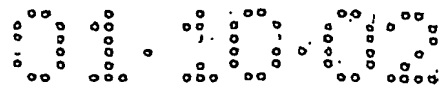
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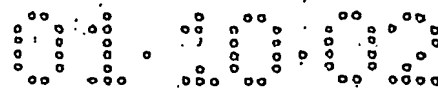
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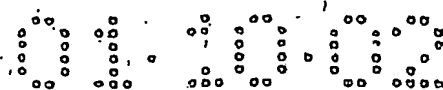
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<210> 271

<211> 2972

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 271

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126

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<210> 272

<211> 2972

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 272

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<10> 273

<11> 6101

<12> DNA

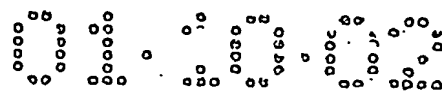
<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 273

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<211> 6101

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<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 274

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<220>

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<221> unsure

<222> (289, 833, 1773, 2369, 2374, 2386)

<223> unknown base

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<210> 276

<211> 2425

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<220>

<221> unsure

<222> (40, 52, 57, 653, 1593, 2137)

<223> unknown base

01.20.02

572

<400> 276

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<210> 277

<211> 2359

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 277

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01:00:02

533

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<210> 278

<211> 2359

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<220> 278

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<211> 2474

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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010000

935

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<211> 2474

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 280

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<211> 6175
<212> DNA
<213> Artificial Sequence

<220>
<223> chemically treated genomic DNA (Homo sapiens)

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532

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<211> 6175

<212> DNA

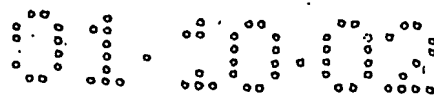
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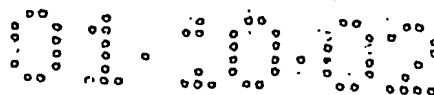
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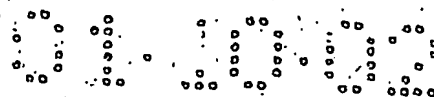
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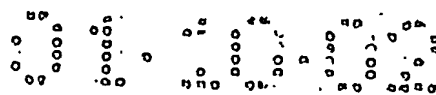
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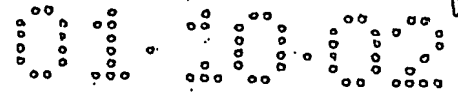
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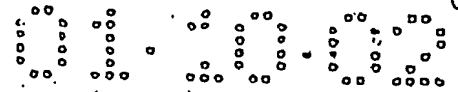
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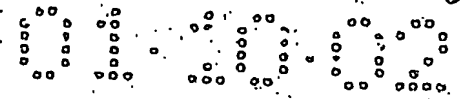
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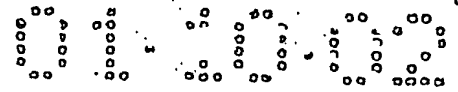
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<211> 2526

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<210> 294

<211> 2526

<212> DNA


<213> Artificial Sequence

<220>


<223> chemically treated genomic DNA (Homo sapiens)

<400> 294

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213



212

 $\langle 213 \rangle$

220

$\langle 220 \rangle$
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100

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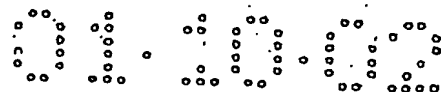
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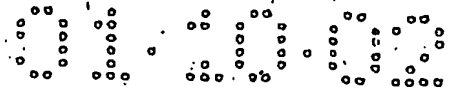
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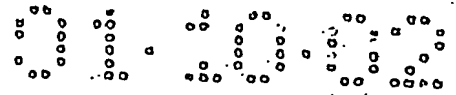
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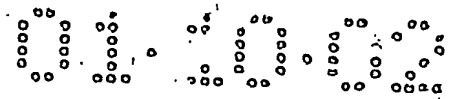
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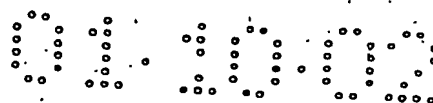
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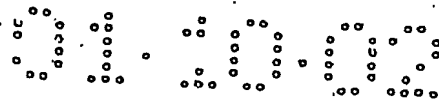
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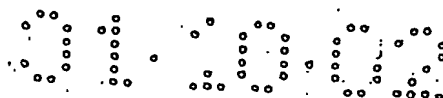
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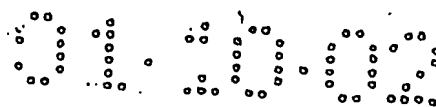
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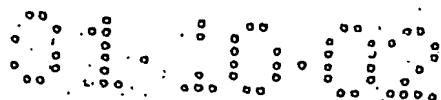
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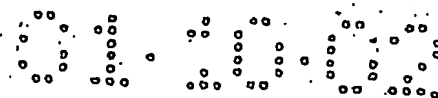
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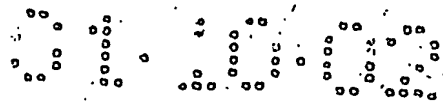
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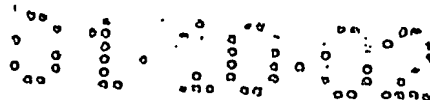
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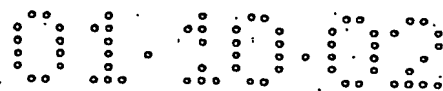
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<211> 5759

<212> DNA

<213> Artificial Sequence

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<223> chemically treated genomic DNA (Homo sapiens)

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<211> 5387

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<220>
<223> chemically treated genomic DNA (Homo sapiens)

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<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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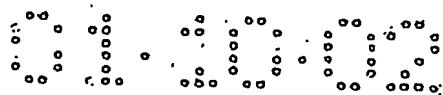
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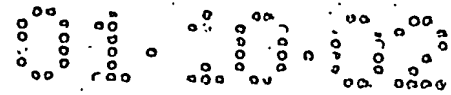
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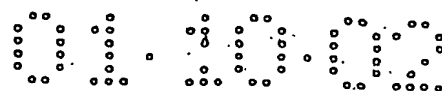
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<210> 318

<211> 5559

<212> DNA

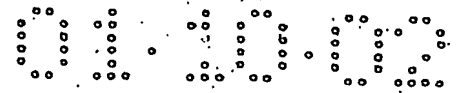
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<220>

<223> chemically treated genomic DNA (Homo sapiens)

<224> 318

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615

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<211> 3476

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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<211> 3476

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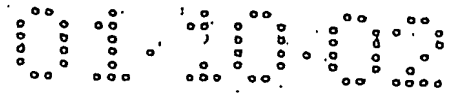
<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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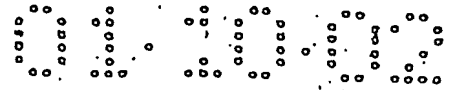
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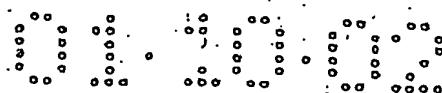
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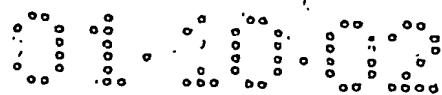
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<210> 325

<211> 3091

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<220>

<221> unsure

<222> (2411, 2632, 2656)

<223> unknown base

<400> 325

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<210> 326

<211> 3091

<212> DNA

<213> Artificial Sequence

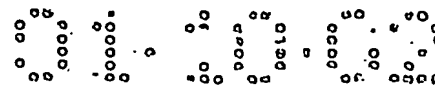
<220>

<223> chemically treated genomic DNA (Homo sapiens)

<220>

<221> unsure

<222> (436, 460, 681)



<223> unknown base

<400> 326

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<210> 327

<211> 3083

<212> DNA

<213> Artificial Sequence

<220>

01:00:00 629

<223> chemically treated genomic DNA (Homo sapiens)

<400> 327

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<210> 328

<211> 3083

<212> DNA

<213> Artificial Sequence

<220>

01:00:00 6 10

<223> chemically treated genomic DNA (Homo sapiens)

<400> 328

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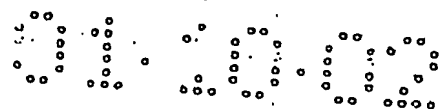
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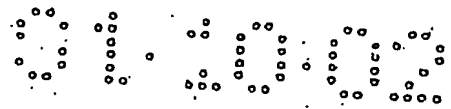
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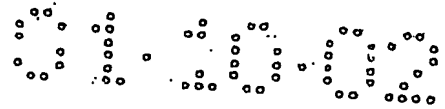
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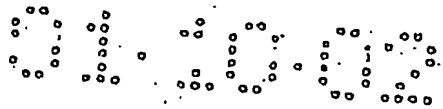
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641

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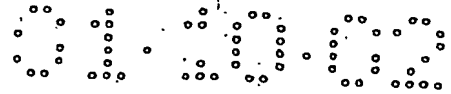
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12

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